DNA Typing for the Identification of Korean War Victims

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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

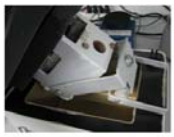
- Complete demineralization

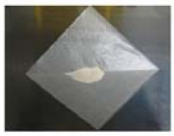
- DNA extraction using silica columns

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.

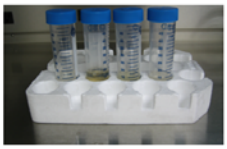
Irradiation with UV light

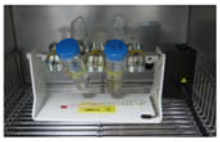




- Cleaned bone sample was powdered using 6750 Spex CertiPrep Freezer/Mill (SPEX CertiPrep, NJ)

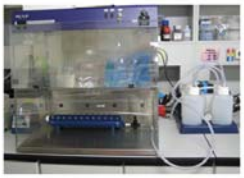
Complete Demineralization





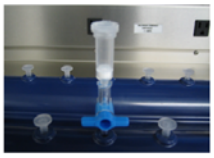
- **Demineralization solution**
  - 0.5 g bone power
  - 15 ml of 0.5 M EDTA and 0.5% SDS
  - 3 mg Proteinase K
- **Incubation**
  - 48 hours at 56℃ in dry incubator
  - 1 hour after additional treatment of 3 mg of Proteinase K

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

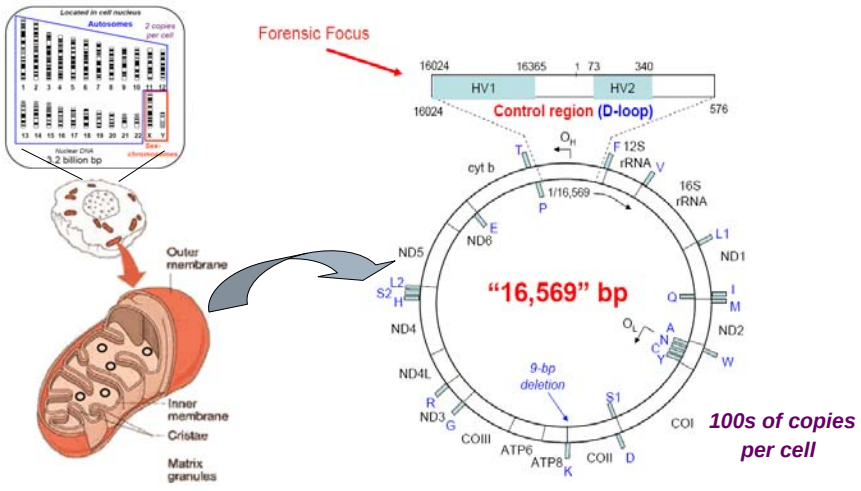
- Quantifiler® Human DNA Quantification kit (Applied Biosystems)
- Applied Biosystems 7500 Real-time PCR system

	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

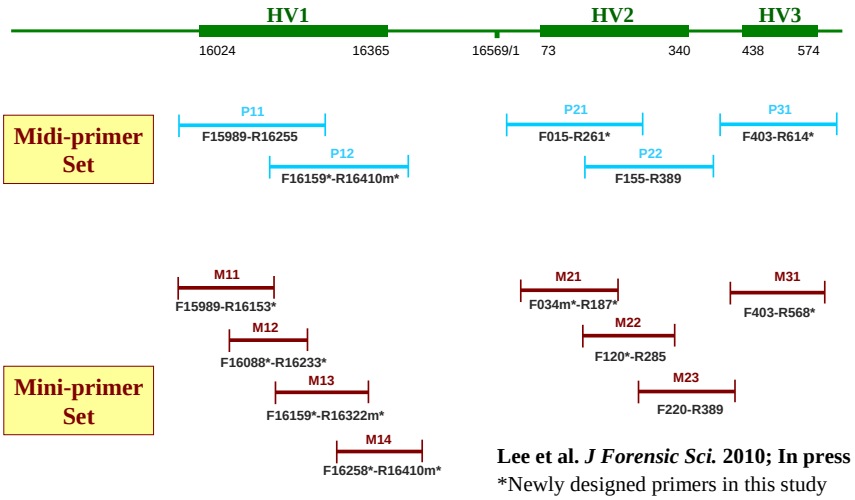
*DNA was extracted using the QIAquick® PCR purification kit after incomplete demineralization of bone powder by small-volume high-concentration of EDTA.

Sequence and Quality Analyses of mtDNA from Old Skeletal Remains

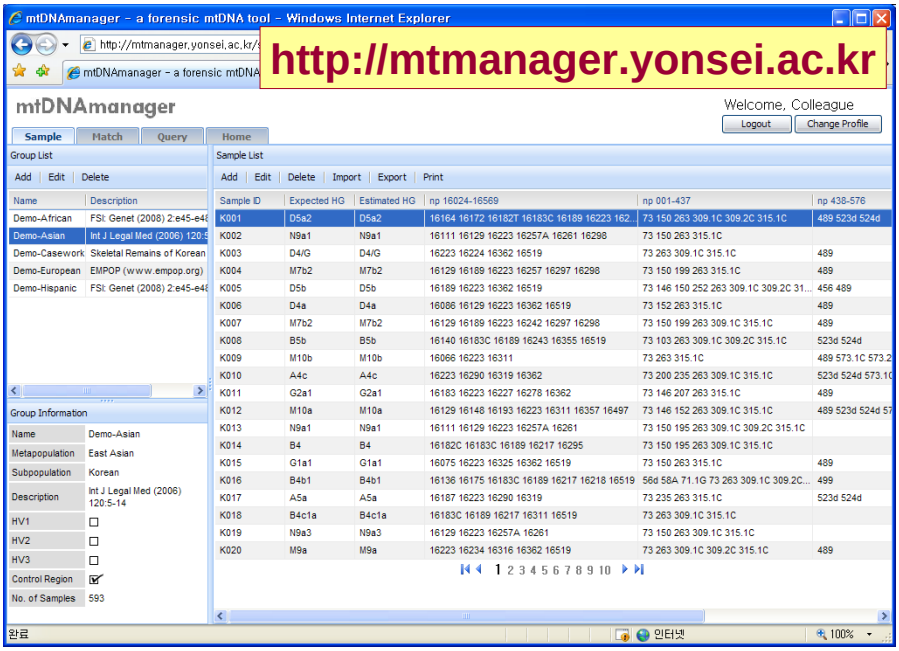
Mitochondrial DNA (mtDNA)



Primers for Amplification of mtDNA



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

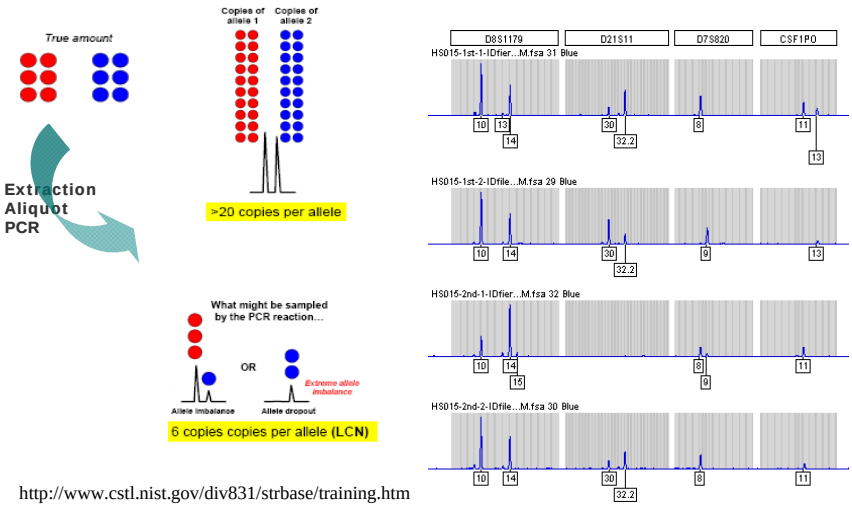


STR Genotyping of DNA from Old Skeletal Remains

PCR amplification for STR genotyping

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

Stochastic Effect on Autosomal STR Typing



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

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

- Independant set up of PCR
  - 1st amplification

    PCR master mix I for 1st DNA extract

    PCR master mix II for 2nd DNA extract

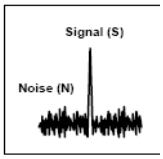
  - 2nd amplification

    PCR master mix III for 1st DNA extract

    PCR master mix IV for 2nd DNA extract

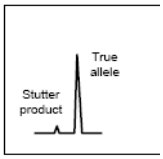
Scoring of STR Alleles

- Peak detection threshold
  - 75 ~ 200 RFU



Signal > 3x sd of noise

- Cut-off minor allele
  - Under 15% of peak height of major peak

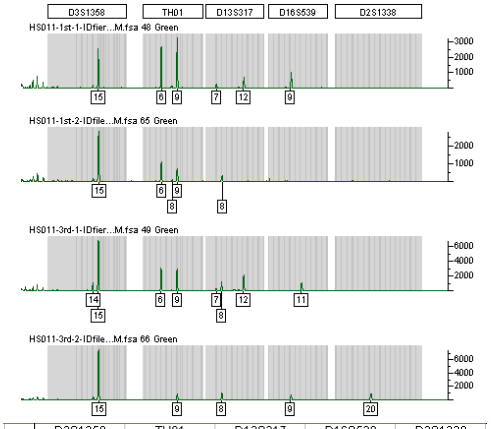


Stutter location above 15%

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

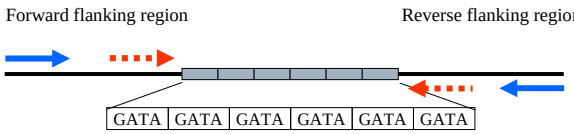
http://www.cstl.nist.gov/div831/strbase/training.htm

Scoring of STR Alleles



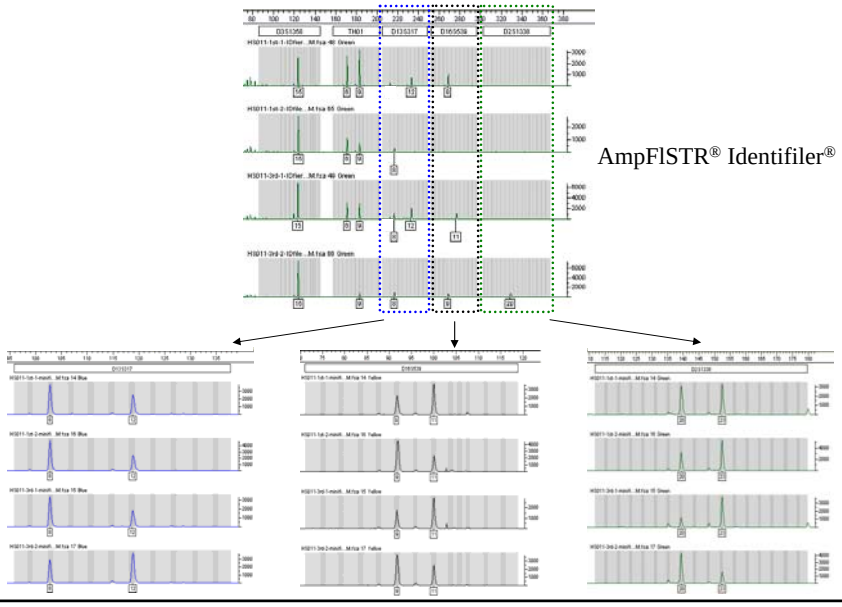
	D3S1358	TH01	D13S317	D16S539	D2S1338
1-1	**15**	**6,9**	7,**12**	**9**	
1-2	**15**	**6 9**	**8**		
3-1	14,**15**	**6,9**	7,8,**12**	**11**	
3-2	**15**	**9**	**8**	**9**	**20**
	15	**6,9**	**8**		

Size-reduced Amplicon of mini-STRs



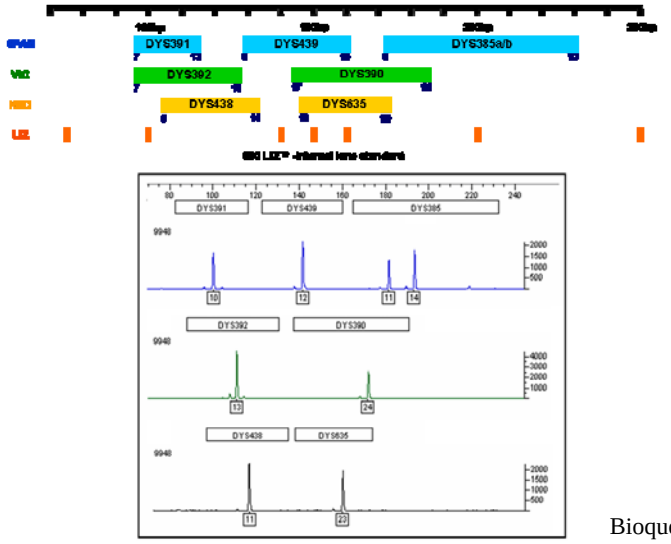
- Advantages of mini-STRs
  - Retains same marker information (database compatibility)
  - Uses highly polymorphic STR loci (high discriminatory power)
- AmpFlSTR® MiniFiler™ was used as a complement to the AmpFlSTR® Identifiler®
- Y-miniplex plus as a complement to the AmpFlSTR® Yfiler™
- In-house miniplex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

AmpFlSTR® MiniFiler™



AmpFlSTR® Identifiler®

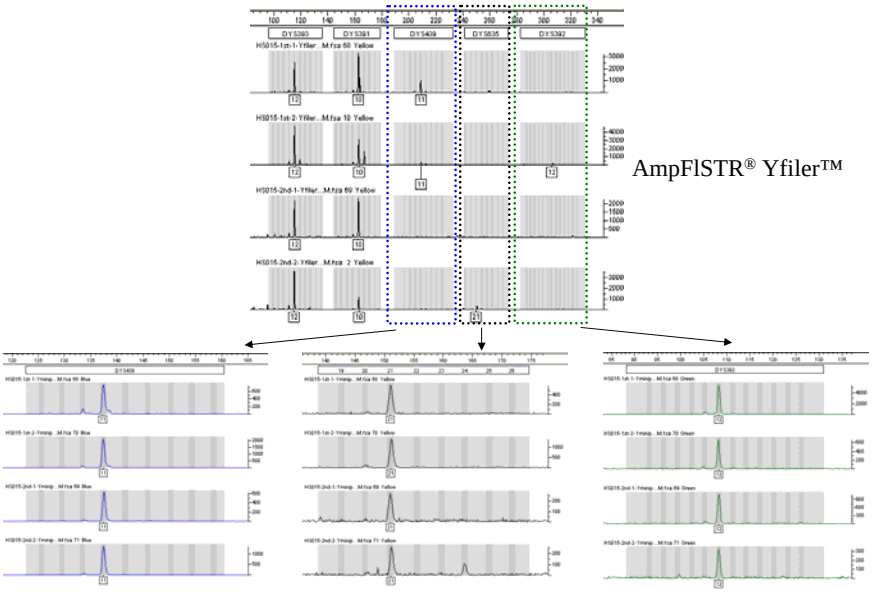
Y-miniplex plus (In-house)



Bioquest *Inc*

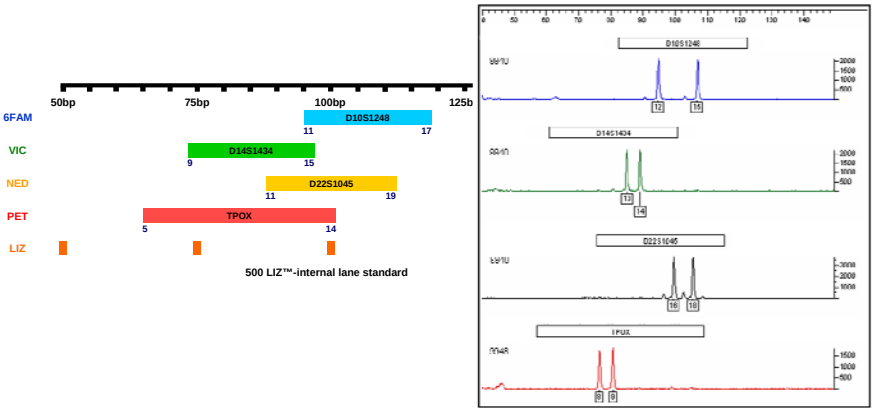
http://forensic.yonsei.ac.kr/protocols.html

Y-miniplex plus



AmpFlSTR® Yfiler™

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 AmpFℓSTR® Identifiler® Kit, AmpFℓSTR® MiniFiler™ Kit, NC01plus, AmpFℓSTR® YFiler™ 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	
		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

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, ¼ and ½

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
D21S11	***30***	29-30	***0.924***	***1.084***	***1.135***
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
TH01	***9***	7-9	***0.733***	***0.873***	***0.931***
D13S317	9-10	9-10	8.854	8.854	8.854
D16S539	12-13	9-10	0.250	0.250	0.250
D2S1338	***22***	22	***336.985***	***31.200***	***22.756***
D19S433	13.2-14	15.2-16.2	0.250	0.250	0.250
vWA	***19***	15-19	***3.124***	***3.345***	***3.363***
TPOX	-	8-11	-	-	-
D18S51	11-15	13-15	0.922	0.922	0.922
D5S818	***11***	9-10	***0.250***	***0.420***	***0.466***
FGA	***23***	23-25	***1.435***	***1.625***	***1.661***
Cummulative LR			2,996.3	791.3	734.3

The STR loci and alleles which have possibility of allele dropout, and their LRs 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 (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.
- To ensure data quality from degraded samples, a redundant approach to data generation and analysis was employed.

Thank you for your attention!

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