

DNA Typing Strategy for the Identification of Old Skeletal Remains

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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 from Old Skeletal Remains

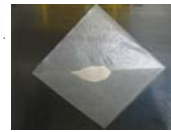
- Low copy number (LCN) DNA
 - Highly degraded DNA
 - Presence of PCR inhibitors
-

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



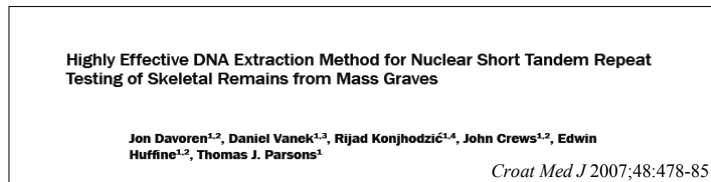
- Cleaned bone sample was powdered using 6750 Spex CertiPrep Freezer/Mill (SPEX CertiPrep, NJ)

DNA Extraction from Old Skeletal Remains

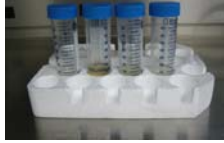
- Complete demineralization



- Large-scale silica-based column extraction



Complete Demineralization



□ Demineralization solution

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

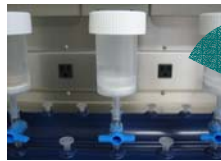
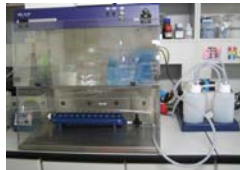


□ Incubation

- 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



50 µl 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

Quantification Using Real-time qPCR

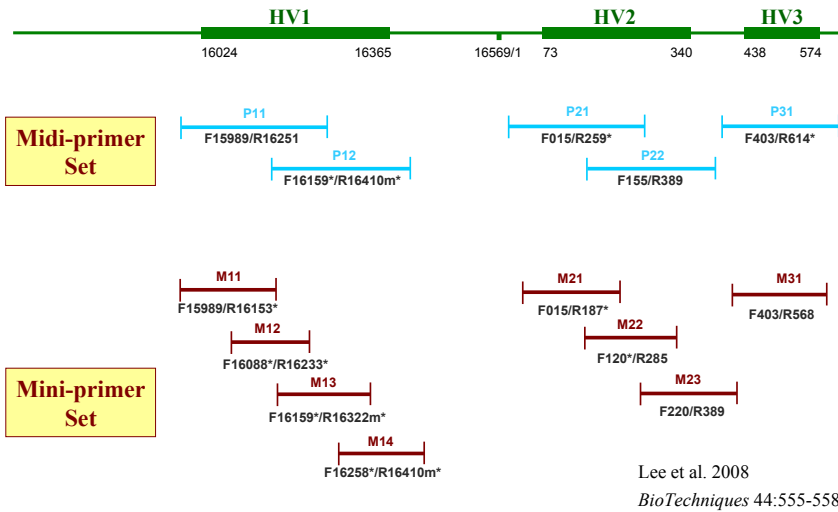
- ❑ Quantifiler® Human DNA Quantification kit
- ❑ Applied Biosystems 7500 Real-time PCR system

	Small-scale* (Yang et al. 1998)	Large-scale
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

Primers for Amplification of mtDNA

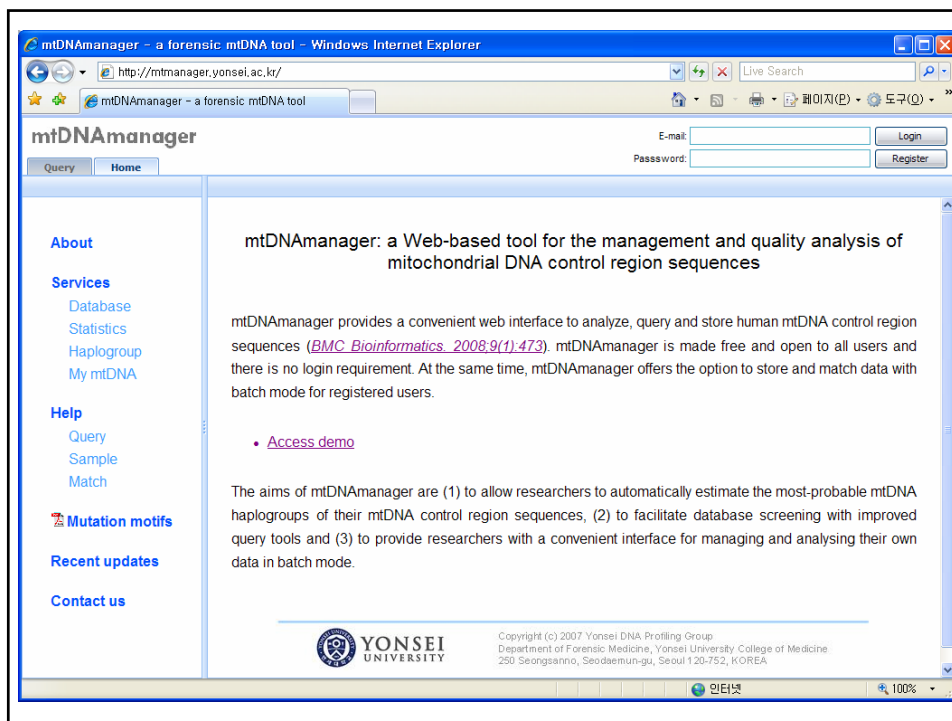


Detection of mtDNA Sequence Errors

The screenshot shows a Science magazine article titled "Problems in FBI mtDNA Database" by D. Kennedy. The article discusses issues with the FBI's mtDNA database, specifically mentioning the "African-American" database and the detection of artificial combinations of mtDNA segments. The article is dated September 2004.

mtDNA database - USA.AFR.000942

HV1	HV2
16126-16187-16189-16223-16264	73-249d-263-290d-291d
16270-16278-16293-16311-16519	309.1C-315.1C-489
African haplogroup L1b	Asian haplogroup C1



mtDNAManager's Open Database

Metapopulation	Subpopulation	No. of sequences	References
African	African	1148	Forensic Sci Commun (2002) 4 <i>Online</i>
	Kenyan	100	Int J Legal Med (2004) 118:294-306
	U.S. African	140	Forensic Sci Int: Genetics (2008) 2(3):e45-48
West Eurasian	Caucasian	1655	Forensic Sci Commun (2002) 4 <i>Online</i>
	Austrian	273	Forensic Sci Int (2007) 166:164-175
	Finnish	200	Forensic Sci Int (2007) 172:171-178
	German	313	Forensic Sci Int (2007) 172:218-224 Int J Legal Med (2006) 120:310-314
	Hungarian	416	Int J Legal Med (2007) 121:377-383
East Asian	East Asian	753	Forensic Sci Commun (2002) 4 <i>Online</i>
	Japanese	211	Int J Legal Med (2003) 117:218-225
	Korean	593	Int J Legal Med (2006) 120:5-14
Oceanian	-	-	-
Admixed	Hispanic	686	Forensic Sci Commun (2002) 4 <i>Online</i>
	Dubaian	249	Forensic Sci Int: Genetics (2008) 2(1):e9-10
	U.S. Hispanic	253	Forensic Sci Int: Genetics (2008) 2(2):e19-23 Forensic Sci Int: Genetics (2008) 2(3):e45-48
	Venezuelan	100	Forensic Sci Int: Genetics (2008) 2(4):e61-64
Total		7090	

Automatic Estimation of Haplogroups

- Along with the data import, simultaneous estimation of the most-probable mtDNA haplogroup is carried out.

Sample List

Sample ID	Expected HG	Estimated HG	rp 16224-16559	rp 001-437	rp 430-576	Comments	Description
#D5	T1	T1	16126 16163 16186 16189 16204 16519	73 195 263 309.1C 315.1C			T1
#D6	T2	T2	16126 16204 16296 16519	73 150 200 263 315.1C			T2
#E1	H11a	H11a	16292 16211	142 195 263 309.1C 315.1C			H*
#E2	H	H	16291 16519	263 315.1C			H*
#E3	H	H	16293 16271 16519	263 309.1C 315.1C			H*
#E4	H1b	H1b	16189 16398 16362 16819	263 309.1C 315.1C	523d 526d		H1b
#E5	X2	X2	16134 16103C 16109 16223 16270 16519	73 195 263 309.1C 309.2C 315.1C			X2d
#E6	J1c	J1c	16069 16126 16201 16265 16319	73 195 226 263 295 309.1C 315.1C	462 489 524.1A 524.2C...		J1c
#F1	H	H	16234 16293 16519	263 315.1C 315.2C			H*
#F2	VV	VV	16223 16292 16290 16519	73 119 189 195 204 207 263 315.1C			VV
#F3	K1a	K1a	16222 16224 16311 16362 16519	73 114 263 309.1C 315.1C	497		K1a
#F4	T1a	T1a	16126 16163 16186 16189 16204 16519	73 152 195 263 309.1C 315.1C			T1a
#F5	H5	H5	16260 16304	263 309.1C 315.1C	456		H5
#F6	U5a	U5a	16192 16256 16270 16299	73 142 263 315.1C			U5a*
#F0	HV0	HV0	16153 16290	72 93 195 263 315.1C			HV0
#G2	H	H	16311	263 309.1C 315.1C	523d 526d		H*
#G3	H	H	16325 16819	263 309.1C 315.1C			H*
#G4	H5	H5	16304	263 309.1C 309.2C 315.1C	456		H5
#G5	T1	T1	16126 16163 16186 16189 16204 16519	73 195 263 315.1C			T1
#G6	C	C	16093 16223 16234 16288 16290 16327 16518...	73 249d 263 309.1C 315.1C	499		C

Haplogroup-specific Mutation Motifs

Control Region Mutation motifs for more than 400 mtDNA haplogroups

mtDNA Haplogroup Specific Control Region Mutation Motifs

African Haplogroup	HV2	HV3 etc.
L0a	16126-16148-16172-16187-16188G-16189-16223-16230-16311-16320	93-183-189-230-247 328a-324d
L0a1	16126-16148-16168-16172-16187-16188G-16189-16223-16230-16311-16320	93-183-189-230-247 328a-324d
L0a1a	16126-16148-16168-16172-16187-16188G-16189-16223-16230-16311-16320	93-183-189-230-247 328a-324d
L0a2	16126-16148-16172-16187-16188G-16189-16223-16230-16311-16320	93-183-189-230-247 328a-324d
L0a3	(16129)-(16179)-16189-16223-16230-16243-16311	189-247
L0a4	16196-16187-16189-16223-(16230)-16278-16311-16327	189-247 26988
L0b	16172-16187-16189-16209-16214-16223-16230-16278-16294G-16311	146-189-191-198-207-247
L1b	16126-16187-16189-16223-16294-16278-16278-16311	182-183T-193-247 337, 329a-324d
L1b1	16126-16187-16189-16223-16294-16278-16278-16294-16311	(182)-183T-(193)-247 337, 329a-324d
L1c	(16129)-16189-(16223)-16278-16311	133-182-186A-189C-247d-248b-316 328a-324d
L1c1	16129-16189-(16223)-16278-16303-16390	(133)-182-186A-189C-247d-248b-316 328a-324d
L1c2	16129-16187-16189-(16223)-16265C-16278-16286G-16294-16311-16340	133-133T-182-186A-189C-198-247-316 328a-324d

Quality Analysis of mtDNA Sequences

Clear key diagnostic mutations

Accompanying mutations for Expected HG

Sample ID	Expected HG	Estimated HG	np 16024-16569	np 001-437	np 436-576	Comments	Description
HD5	T1	T1	16126 16294 16296 16519	73 195 263 309.1C 315.1C			T1
HD6	T2	T2	16126 16294 16296 16519	73 150 200 263 315.1C			T2
HD1	HT1a	HT1a	16292 16211	142 195 263 309.1C 315.1C			HT
HD2	H	H	16291 16519	263 315.1C			HT
HD3	H	H	16293 16271 16519	263 309.1C 315.1C			HT
HD4	HTb	HTb	16189 16398 16362 16519	263 309.1C 315.1C	523d 524d		HTb
HD5	X2	X2	16134 16183C 16189 16223 16270 16519	73 195 263 309.1C 309.2C 315.1C			X2d
HD6	J1c	J1c	16069 16126 16201 16265 16319	73 195 226 263 295 309.1C 315.1C	462 489 524.1A 524.2C...		J1c
HD1	H	H	16234 16293 16519	263 315.1C 315.2C			HT
HD2	VV	VV	16223 16292 16296 16519	73 119 189 195 204 207 263 315.1C			VV
HD3	K1a	K1a	16222 16224 16311 16362 16519	73 114 263 309.1C 315.1C	497		K1a
HD4	T1a	T1a	16126 16163 16186 16189 16294 16519	73 152 195 263 309.1C 315.1C			T1a
HD5	H5	H5	16260 16304	263 309.1C 315.1C	456		H5
HD6	USa	USa	16192 16256 16270 16299	73 142 263 315.1C			USa1
HD1	HV0	HV0	16153 16290	73 93 195 263 315.1C			HV0
HD2	H	H	16311	263 309.1C 315.1C	523d 524d		HT
HD3	H	H	16325 16519	263 309.1C 315.1C			HT
HD4	H5	H5	16304	263 309.1C 309.2C 315.1C	456		H5
HD5	T1	T1	16126 16163 16166 16169 16294 16519	73 195 263 315.1C			T1
HD6	C	C	16093 16223 16234 16288 16296 16327 16518	73 249d 263 309.1C 315.1C	489		C

Detection of Possible mtDNA Errors

N9a1: 16129-16223-16257A-16261-150

Sample ID	Expected HG	Estimated HG	np 16024-16569	np 001-437	np 436-576	Comments
Demo-01	D4b1	D4b1	16223 16319 16362	73 150	489 523d 524d	
Demo-02	N9a1	N9a1	16129 16223 16257A 16261	73 152 263 309.1C 315.1C		
Demo-03	A5b	A5b	16126 16223 16235 16290 16319 16519	73 235 263 315.1C	523d 524d	
Demo-04	D4a3	D4a3	16129 16223 16249 16266 16304 16362 16519	73 152 263 309.1C 315.1C	489	
Demo-05	F1b	F1b	16129R 16182C 16183C 16189 16232A 16249	73 152 249d 263 315.1C	523d 524d	16129R
Demo-06	A4	A4	16223 16290 16319 16362	73 146 152 200 235 263 309.1C 315.1C	523d 524d	
Demo-07	B5b	B5b	16140 16183C 16362	103 263 315.1C	523d 524d	
Demo-08	D4/G	D4/G	16223 16260 16292	73 146 263 309.1C 315.1C	489	
Demo-09	A5a	A5a	16187 16223 16290 16519	73 146 195 235 263 309.1C 309.2C 315.1C	523d 524d	
Demo-10	D4/J	D4/J	16184 16223 16311 16362			
Demo-11	M9a	M9a	16223 16234 16316 16362			
Demo-12	B4c1a	B4c1a	16086 16183C 16362 16519	73 263 309.1C 315.1C	523d 524d	
Demo-13	M7b2	M7b2	16129 16185	73 150 152 199 263 309.1C 315.1C	489 573.1C	
Demo-14			16223 16362T	73 263 315.1C	489	16362T
Demo-15	M7a	M7a	16209 16223	73 263 309.1C 315.1C	489 523d 524d	
Demo-16	F1a1	F1a1	16129 16162 16172 16304 16519	64 73 249d 263 309.1C 315.1C	523d 524d	
Demo-17	F2*	F2*		73 195 200 235 249d 263 309.1C 315.1C	459d	
Demo-18	G3a	G3a	16223 1627	73 143 152 204 263 315.1C	489	
Demo-19	A	A	16179 16223	73 235 263 309.1C 315.1C	523d 524d	
Demo-20			16066 16223	73 103 204 263 315.1C	489	

16319 missed out?

16362C?

16311 missed out M10b?

B5b?

Artificial recombination?

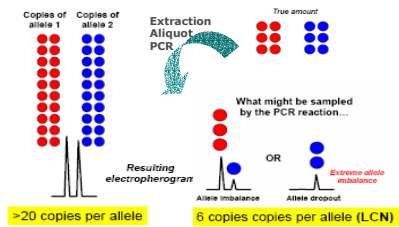
STR Genotyping of DNA from Old Skeletal Remains

STR Typing Using Low Copy Number DNA

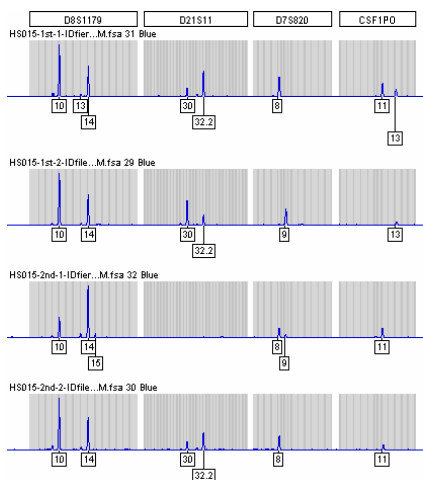
LCN DNA

- Allele drop out
- Allele drop-in

Stochastic effect



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



PCR Strategy for LCN DNA

“LCN interpretation rule”

Replicate analyses with duplicate results prior to reporting alleles

□ 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

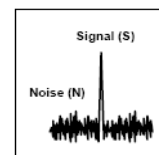
PCR master mix III for 1st DNA extract

PCR master mix IV for 2nd DNA extract

Scoring of STR Alleles

□ Peak detection threshold

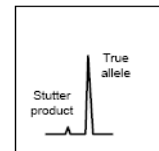
- 75 ~ 200 RFU



Signal > 3x sd of noise

□ Cut-off minor allele

- Under 15% of peak height of major peak

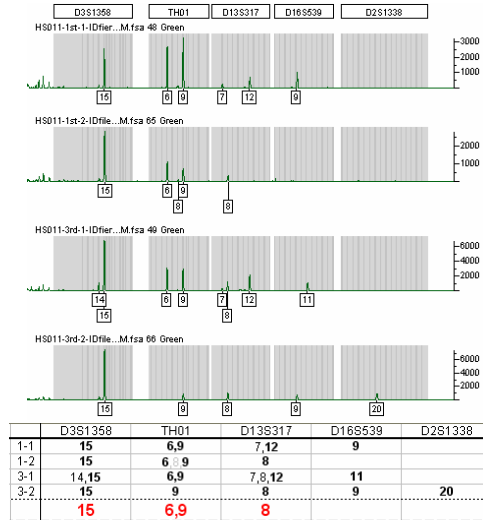


Stutter location above 15%

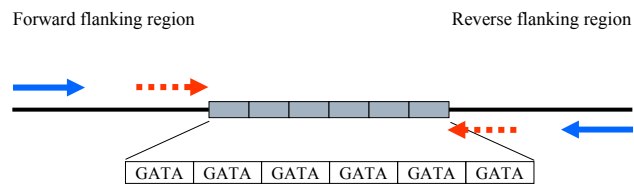
- ### □ An allele cannot be scored unless it is observed at least tree times in four PCR reactions

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

Scoring of STR Alleles

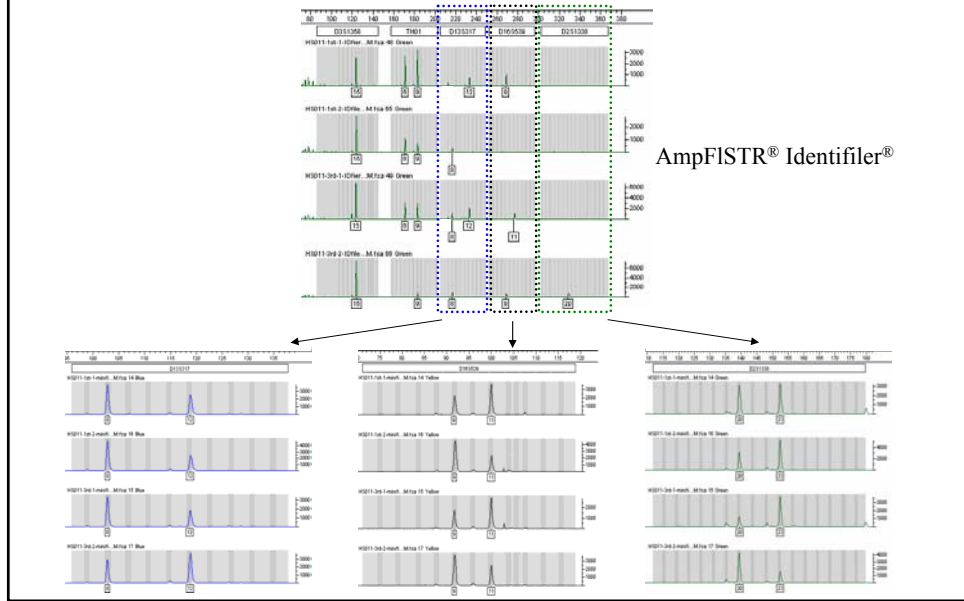


Size-reduced Amplicon of mini-STRs

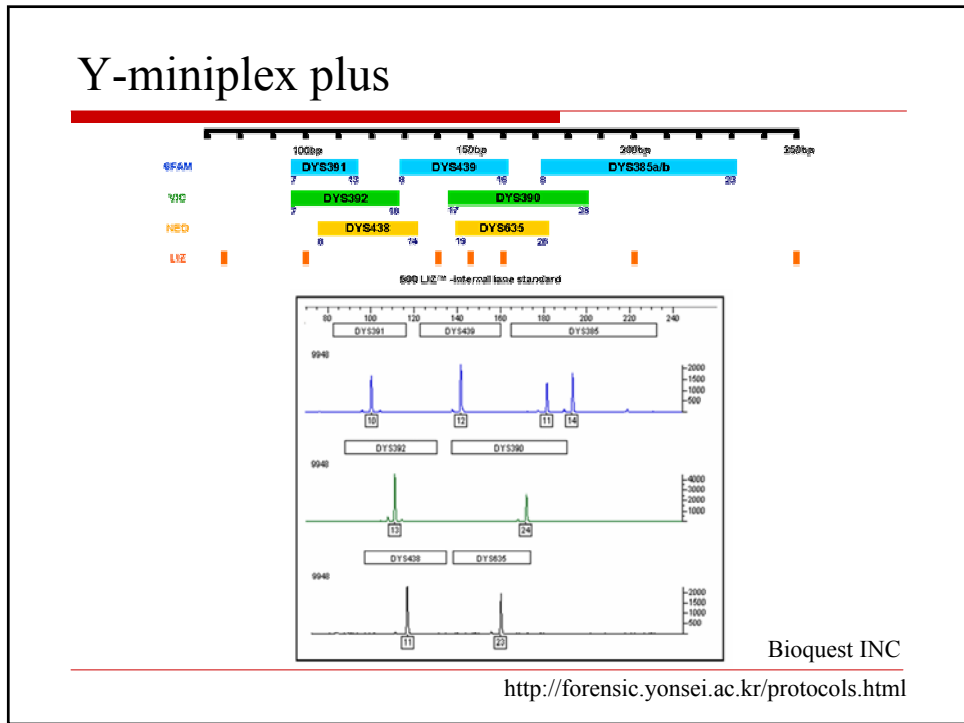


- AmpFISTR® MiniFiler™ was used as a complement to the AmpFISTR® Identifier®
- Y-miniplex plus as a complement to the AmpFISTR® Yfiler™
- In-house miniplex NC01 plus was used to increase the discrimination capacity of the system

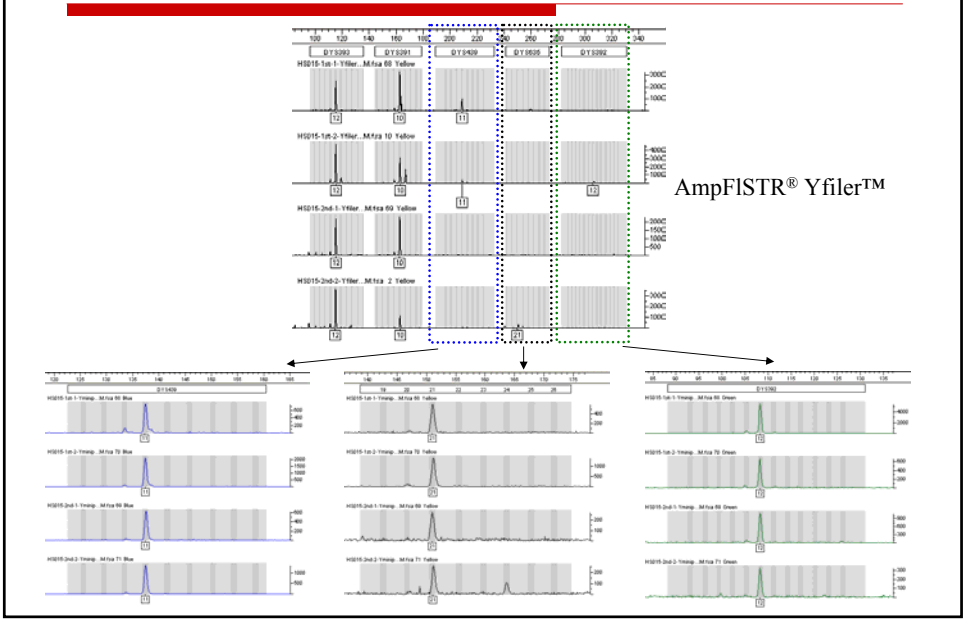
AmpFISTR® MiniFiler™



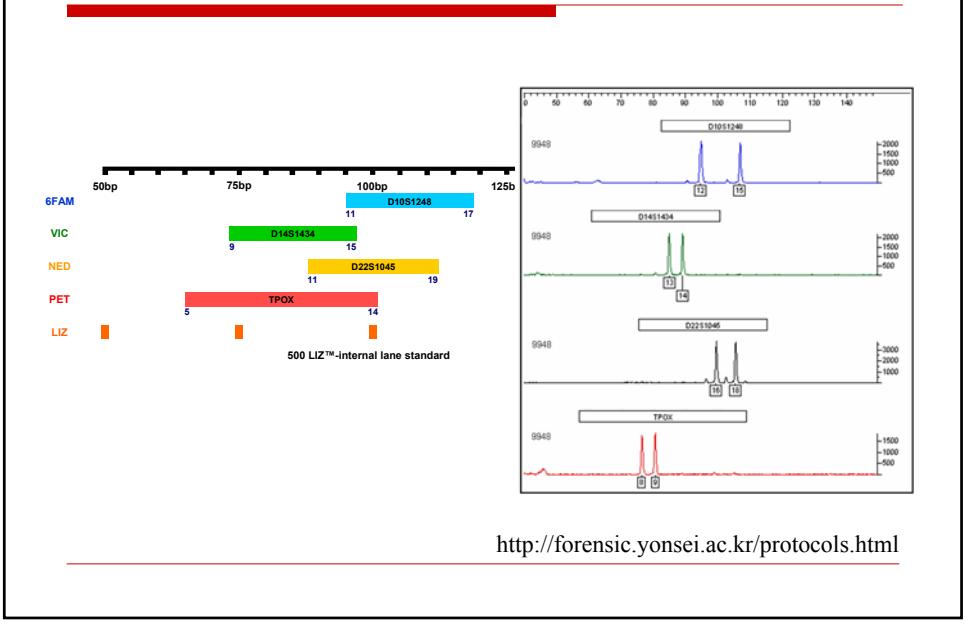
Y-miniplex plus



Y-miniplex plus



In-house NC01 plus



Improved STR Typing Results

- Mean number of successfully genotyped STR loci was calculated using AmpF ℓ STR[®] Identifiler[®] Kit, AmpF ℓ STR[®] MiniFiler[™] Kit, NC01plus, AmpF ℓ STR[®] 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	
		AmpF ℓ STR [®] Identifiler [®] Kit	AmpF ℓ STR [®] MiniFiler [™] Kit	NC01 plus	AmpF ℓ STR [®] 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

Thank you for your attention!



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