Genetic Identification of Ancient Korean Remains

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Merits and difficulties of ancient DNA analysis in human genetics

- The analysis of ancient DNA is an ideal way to get a direct grip on the past
- The vanishingly small traces of DNA fragments left behind in old specimens and the ubiquitous nature of contamination DNA make it very difficult to obtain reliable DNA sequence data from most ancient samples

The Nine Criteria by Cooper and Poinar (Science. 2000;18:1139)

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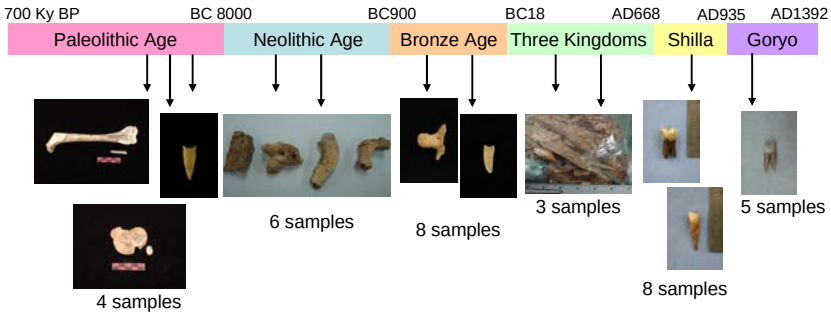
Contamination precautions taken to ensure the reliability of results

- Physical methods that remove the bone surface and UV irradiation that makes DNA unsuitable for PCR
- Isolated laboratory where no post-PCR work has been conducted
- Testing of control extracts in parallel with extracts from old specimens
- Multiple extractions from the same samples at different times
- Quantitation of amplifiable DNA using PCR

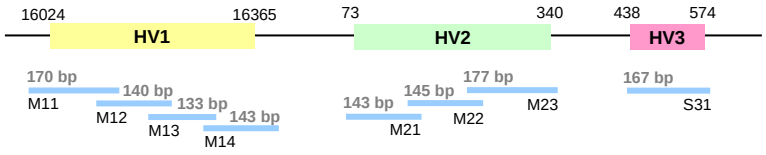
mtDNA analysis from ancient Korean human remains

- 35 museum samples ranged from the Paleolithic age to Goryo dynasty



Control region sequence analysis using small PCR amplicons

- Mitochondrial DNA control region sequences were obtained from 8 small overlapping PCR fragments (133 – 177 bp)



The screening for cross-contamination or sample mix-up was required for assessing authenticity

Self-help guide to avoid errors

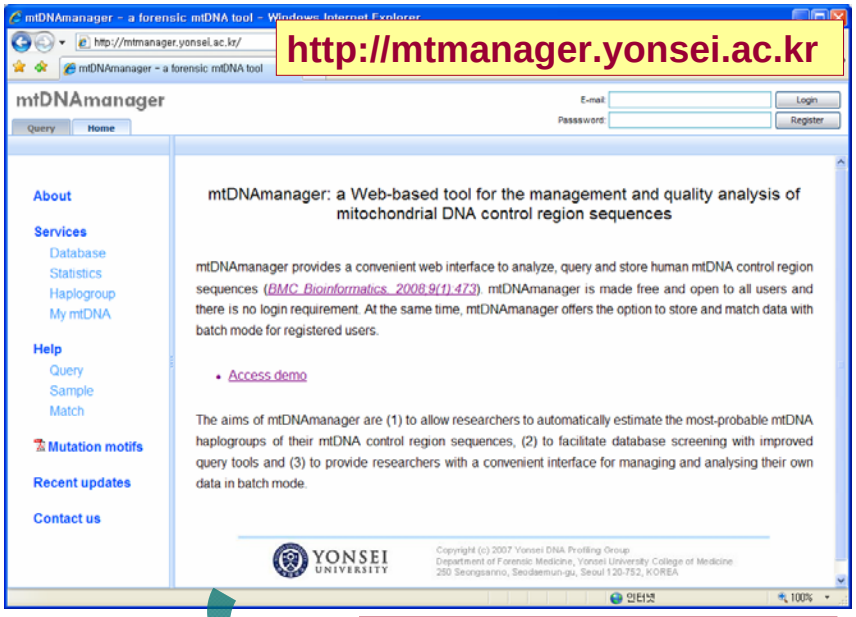
- Attempts to localize the sequence to a part of phylogeny (**haplogroup**). If the haplogroup motif is not fully represented, recheck the relevant positions in the sequence
- Have in mind the **relative mutability of sites**. Be sensitive to rare mutations on different sequence backgrounds in one batch of sequencing
- Look out for incongruence between parts of the sequences which have been obtained in different PCR or sequencing reactions (**artificial recombinants**)

Need to establish a big reliable database with mtDNAs having appropriate haplogroup designation

mtDNAmanager (http://mtmanager.yonsei.ac.kr)

- The goal of mtDNAmanager is to provide a Web-based forensic mitochondrial DNA bioinformatics resource for supporting **data quality control** and generating **reliable frequency estimates** using a new approach based on haplogroup estimation and data comparison with the contents of a given database.

- mtDNAmanager consists of previously reported **high quality mtDNA sequences**, and a set of **bioinformatics tools**, able to automatically characterize newly submitted data by **estimating its haplogroup according to the haplogroup-specific control region mutation motif.**



Poster presentation: #O-1

mtDNA haplogroup determination by the control region mutation motifs

Sample	Relative dating	Haplogroup	Control Region Sequence		
			nt16024-nt16365	nt073-nt340	nt438-nt548
KO-02†	Paleolithic Age	B4b1	16136-16182C-16183C-16189-16217-16284-16357	73-199-202-207-263-309.1C-309.2C-315.1C	499
KO-06†	Three Kingdoms	D4e1	16092-16187-16223-16362	73-94-263-315.1C	489
KO-07	Goryo	F1a1	16129-16162-16172-16304	73-249d-263-309.1C-315.1C	523d-524d
KO-08†	Goryo	D6	16183C-16189-16223-16274-16362	73-263-309.1C-309.2C-315.1C	489
KO-09	Goryo	A5c	16126-16129-16213-16223-16290-16319	73-152-235-263-309.1C-315.1C	
KO-18†	Goryo	N9a1	16129-16189-16223-16257A-16261	73-150-263-309.1C-309.2C-315.1C	
KO-28†	Neolithic Age	B4f	16168-16172-16183C-16189-16217-16249-16266-16325	73-200-257-263-309.1C-315.1C	
KO-29	Neolithic Age	D4c	16223-16245-16362	73-263-315.1C	489
KO-30	Paleolithic Age	G3a	16223-16274-16325-16362	73-143-152-263-309.1C-315.1C	489
KO-32†	Neolithic Age	B4b1	16136-16183C-16189-16217-16284N	73-199-202-207-263-309.1C-315.1C	499
KO-34	Bronze Age	D4	16223-16362	73-152-263-315.1C	489-523d-524d
KO-35	Bronze Age	D4c	16223-16224-16245-16292-16362	73-146-263-315.1C	489

*Determined haplogroups and haplogroup-specific control region mutation motifs are indicated in blue and red, respectively
†Haplotypes which have partial but articulate sequences

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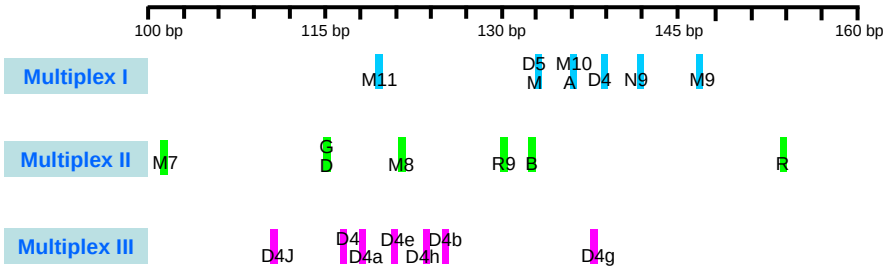
Coding region SNP scoring using three PCR multiplexes

- Small amplicon sizes of the three PCR multiplexes enabled SNP score to be successfully analyzed in old skeletal remains



Lee *et al*. Electrophoresis. 2006;27:4408-4418

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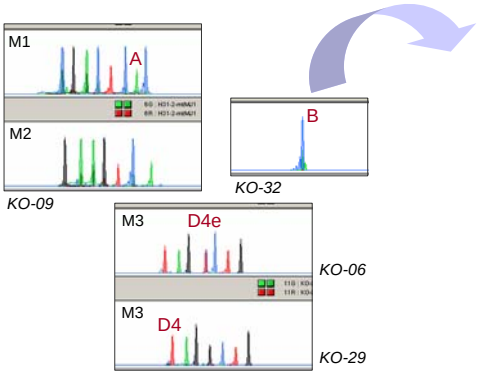
Confirmation of the haplogroup-specific coding region SNPs

- Diagnostic coding region SNPs were confirmed using monoplex SNaPshot, multiplex SNaPshot or sequencing



Sample	Haplogroup	Coding Region SNP
KO-02	B4b1	9bp del
KO-06	D4e1	3010, 14668, 11215
KO-07	F1a1	3970
KO-08	D6	4883, 12654
KO-09	A5c	8794
KO-18	N9a1	5417
KO-28	B4f	9bp del
KO-29	D4c	3010, 14668
KO-30	G3a	4833
KO-32	B4b1	9bp del
KO-34	D4	3010, 14668
KO-35	D4c	3010, 14668

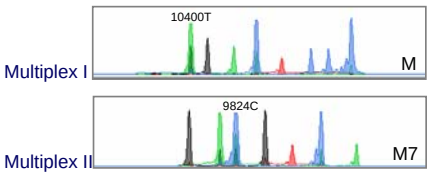
mtDNA haplogroup determination in ancient Korean human remains

- Contamination from the investigators was excluded on the basis of mtDNA sequence comparison results
- 12 of 35 mtDNAs were successfully assigned to appropriate East Asian mtDNA haplogroups or subhaplogroups
  - ✓ No compound haplotype
  - ✓ No mosaic structure
  - ✓ No abnormal mutation

mtDNA analysis in samples from geographically distant region

- A 800 year-old skeletal remain sample was obtained from East Mongolia
- mtDNA haplogroup could be determined by the control region sequence analysis and the coding region SNP analysis
- Haplogroup-directed database comparison was performed in 1192 East Asian mtDNA database

HV1-HV2-HV3 region sequence
16129-**16152**-**16179**-16192-**16223**-16362
73-263-315.1C-**489**



Haplogroup-directed database comparisons to reveal mtDNA origin

- Sequence variations shown in the skeletal remain were detected in the Ulchi of the Lower Amur, not in 1192 East Asian mtDNA database. This supports the sample authenticity

HV1 sequence variation

Skeletal remain found in Mongolia
16129-16152-16179-16192-**16223-16362**
M7d observed in Ulchi
16129-16152-16179-16189-**16223-16362**

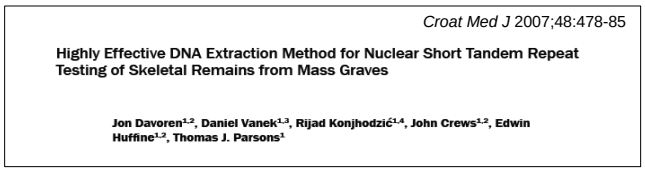
Starikovskaya EB et al. ***Ann. Hum. Genet.*** *(2005) 69:67-89*

New approach for DNA extraction from old skeletal remains

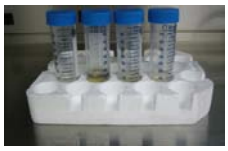
- Complete demineralization

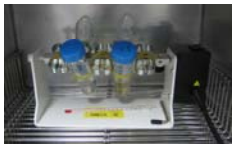

Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 1 (2007) 191–195
FSI GENETICS
Short communication
High efficiency DNA extraction from bone by total demineralization☆
Odile M. Loreille *, Toni M. Diegoli, Jodi A. Irwin, Michael D. Coble, Thomas J. Parsons
Received 24 January 2007; accepted 3 February 2007

- Large-scale silica-based column extraction


Croat Med J 2007;48:478-85
Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves
Jon Davoren[1,2], Daniel Vanek[1,3], Rijad Konjhodzić[1,4], John Crews[1,2], Edwin Huffine[1,2], Thomas J. Parsons[1]

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 columns

2 ml of DNA extract

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

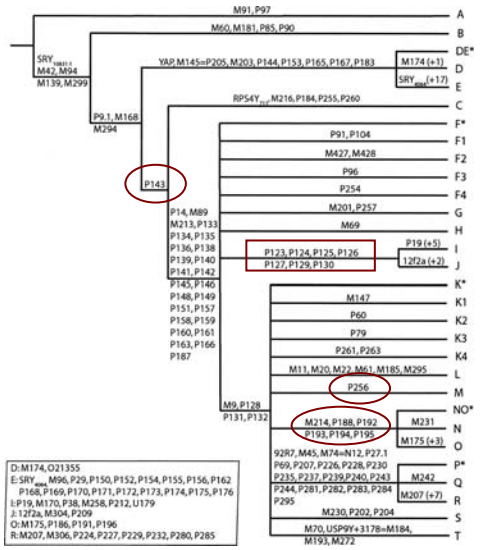
- Concentration of DNA extract
  - QIAamp® DNA Mini column
  - Buffers from QIAquick® PCR purification kit

50 µl of DNA extract

Quantitative RT-PCR comparing different DNA extraction methods for old skeletal remains

Sample	Mass (g)	Method A		Method B		New Method	
		Concentration (pg/µl)	IPC C_T[a]	Concentration (pg/µl)	IPC C_T[a]	Concentration (pg/µl)	IPC C_T[a]
1	0.40	35.0 ± 3.42	28.3 ± 0.04	n.d.[c]	28.0 ± 0.00	114.8 ± 10.20	28.4 ± 0.02
2	0.50	n.d.[c]	27.8 ± 0.01	17.0 ± 3.64	27.8 ± 0.06	212.0 ± 06.19	28.1 ± 0.09
3	0.50	57.2 ± 0.80	28.2 ± 0.06	12.7 ± 1.12	27.8 ± 0.11	054.5 ± 07.86	28.1 ± 0.00
4	0.50	50.3 ± 7.51	27.5 ± 0.04	16.9 ± 3.11	27.5 ± 0.13	100.7 ± 04.62	27.5 ± 0.10
5	0.45	519.4 ± 37.82	27.9 ± 0.13	61.1 ± 4.89	27.7 ± 0.10	825.1 ± 44.46	28.0 ± 0.02
6	0.50	62.6 ± 14.43	28.0 ± 0.05	4.7 ± 3.86	27.8 ± 0.12	118.7 ± 32.71	28.3 ± 0.23
7	0.50	21.5 ± 3.12	27.7 ± 0.14	n.d.[c]	27.8 ± 0.12	034.4 ± 07.86	27.6 ± 0.08
8	0.55	143.2 ± 39.94	27.9 ± 0.19	28.9 ± 0.44	28.0 ± 0.11	156.8 ± 07.55	28.4 ± 0.02
9	0.50	54.9 ± 2.70	28.3 ± 0.11	29.1 ± 10.71	28.6 ± 0.07	112.4 ± 24.15	28.6 ± 0.23
10	0.50	70.7 ± 3.58	28.4 ± 0.11	35.7 ± 1.55	27.9 ± 0.33	327.3 ± 16.58	28.7 ± 0.11
Degraded DNA[b]	5.0×10^{-8}	232.4 ± 73.41	27.5 ± 0.14	91.0 ± 20.27	27.6 ± 0.05	236.8 ± 77.32	27.7 ± 0.15

Major revisions in Y chromosome tree topology



Key changes: P143
Haplogrup C, FT
Not typically found in sub-Saharan African
→ Ancestral C-FT chromosome may have been carried out of Africa very early in the modern human diaspora

Seven mutation
→ IJ clade

Six mutation
→NO clade

P256
M + K-M353/M37 + K-P117/118 = Super M

K-M230 → clade S
K-M70 → clade T

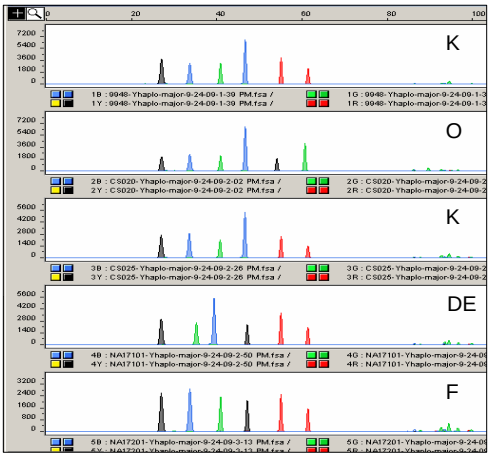
A, E, O, R, J have >40 mutation

Tatiana *et al*. Genome Res. 2008;18:830-838

Y-SNP analysis for highly degraded DNA (under developing)

- Amplicon size < 100
- Subhalogrouping of O

Fine characterization of Korean male lineage



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Korean Mummies

- Danwoong-mirra (550 year ago)

- Youn-mirra (450 yaer ago)

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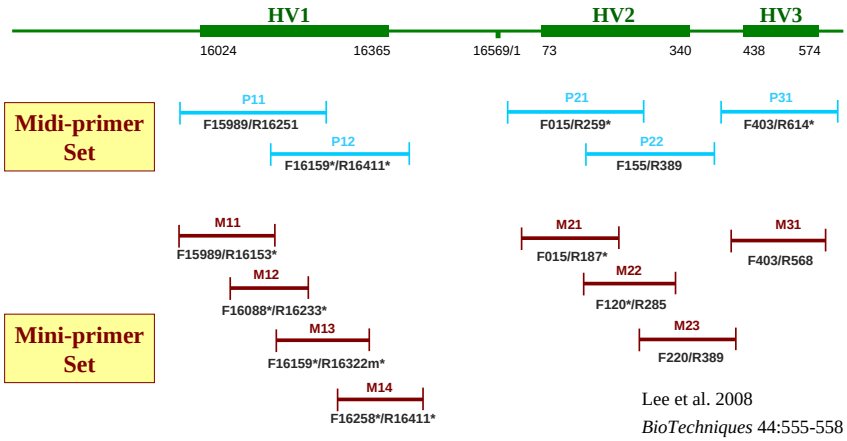
Modified mtDNA PCR primer sets



Lee et al. 2008
BioTechniques 44:555-558

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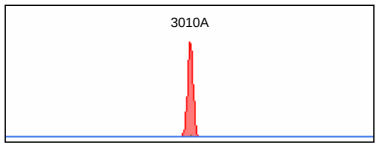
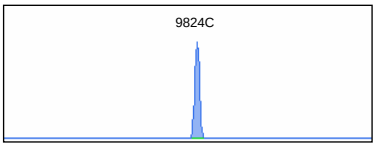
mtDNA control region sequence and haplogroup specific SNP analysis

- Sequence analysis by mtDNAmanager

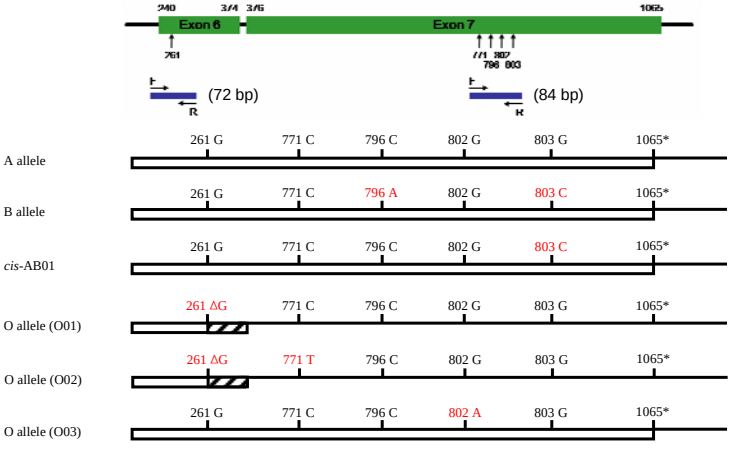
- Danwoong-mirra (D4)



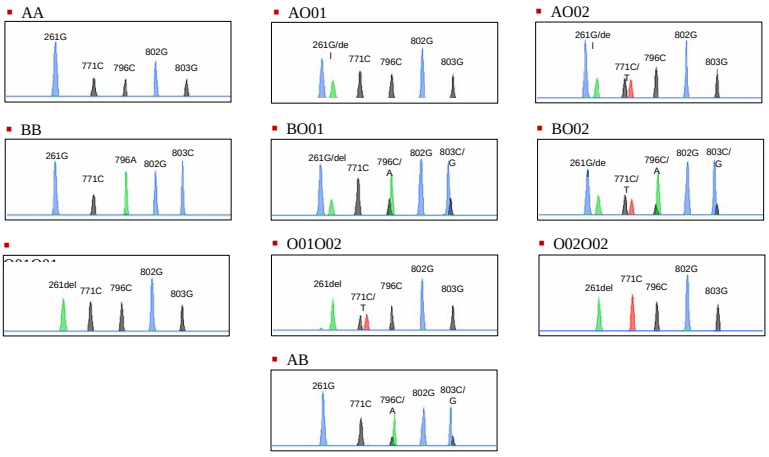
- Youn-mirra (M7)



Selection of SNPs from ABO gene

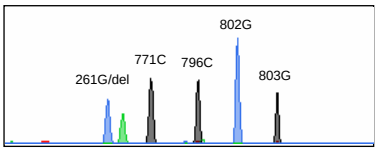


SBE electropherogram of ABO blood type

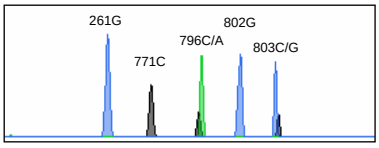


ABO blood type of Korean mummies

- Danwoong-mirra (AO01)



- Youn-mirra (AB)



Concluding Remarks

- Recent advances in DNA extraction techniques and approaches using smaller amplicons have significantly increased the possibility of obtaining DNA profiles from highly degraded skeletal remains.
- In addition, determination of mtDNA and Y chromosomal haplogroups based on worldwide phylogeny has become an additional tool that would be effective and successful in assessing ancient DNA.

Thank you for your attention!

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http://forensic.yonsei.ac.kr

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