A Genetic Investigation of Two Korean Mummies from the Joseon Dynasty

Na Young Kim[1], Hwan Young Lee[1,2], Myung Jin Park[1], Woo Ick Yang[1], Kyoung-Jin Shin[1,2]

[1]Department of Forensic Medicine, Yonsei University College of Medicine
[2]Human Identification Research Center, Yonsei University

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

Korean mummies

- Mummification process
  - Structure of air-tight tombs
- Scientific investigation
  - Causes of death
  - Paleopathological statuses
  - Parasite loads
  - ⋮
- Genetic investigation
  - mtDNA hypervariable (HV) region sequences (in 2003)

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

Development of genetic investigation

- DNA extraction
  - Total Demineralization (*Forensic Sci Int Genet* 2007; 1:191-5)
  - Large scale silica column (*Croat Med J* 2007; 48:478-85)
- Small size amplicon strategy
  - Mini-primer set (*BioTechnique* 2008; 44;555-8)
- mtDNA haplogroup
  - East Asian mtDNA phylogeny (*Hum Mol Genet* 2006; 15:2076–86)
  - mtDNAmanager program (*BMC Bioinformatics* 2008; 9:483)

Danwoong-mirra

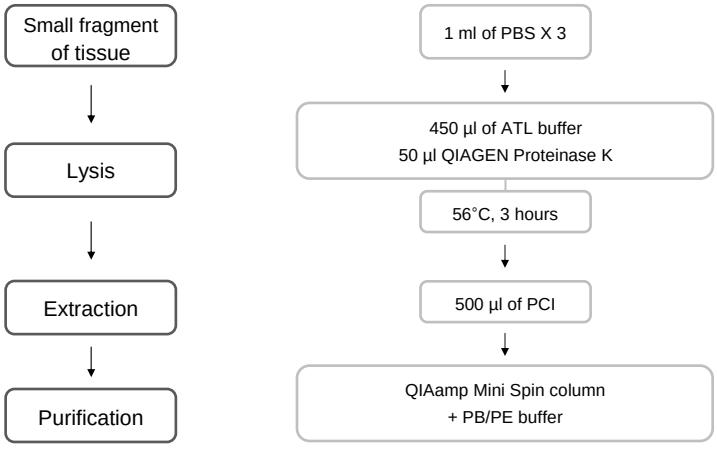
- Discovery of Yangju, Gyeonggi-do, in 2001
- A male child mummy
  : Ages of 4.5 to 6.6 years old
- Dating back to 550 years ago
- First mummy of scientific investigation, in Korea
- Muscles tissue

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

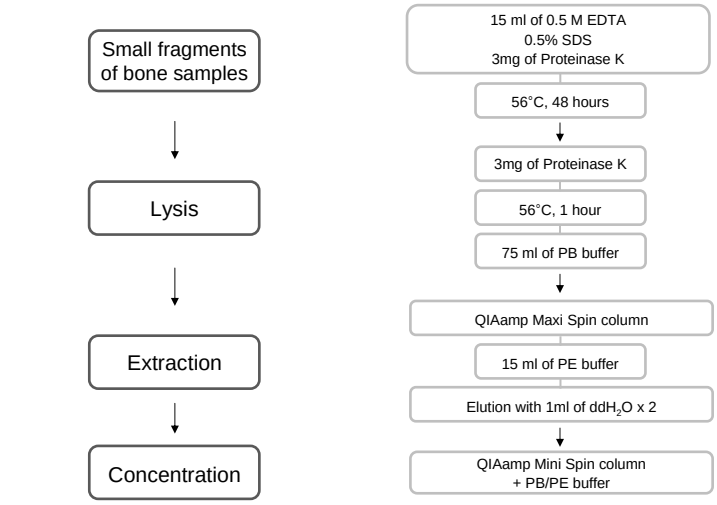
Yoon-mirra

- Discovery of Paju, Gyeonggi-do, in 2002
- A pregnant female mummy
  : Ages of 20 to 30 years old
- Document of death date
  : December of 1566
- Rib bones and liver tissues

DNA extraction from mummified soft tissue



DNA extraction from bone sample

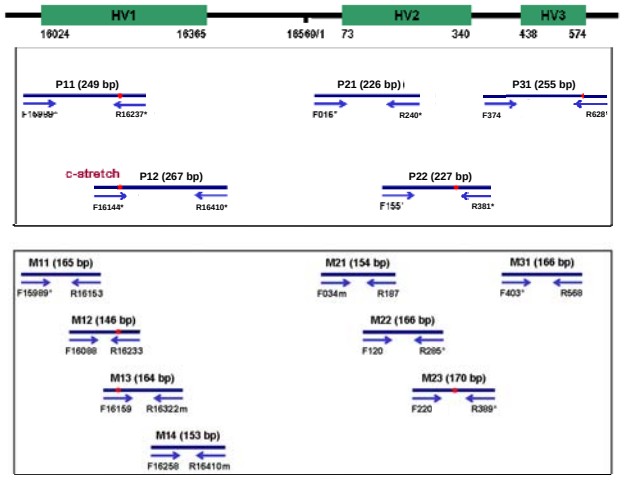


Lee et al. (In press)
Forensic Sci Int Genet

Primer for amplification of mtDNA

In 2003,
AFDIL primer

In 2009,
Mini-primer set



Lee et al. (In press) J Forensic Sci

PCR amplification of mtDNA HV regions

- PCR Mixture

PCR component	volume
dH_20	17.0 µl
Gold ST*R Buffer	2.5 µl
Forward Primer (10 pmol/µl)	1.5 µl
Reverse Primer (10 pmol/µl)	1.5 µl
AmpliTaq Gold (5 U/µl)	0.5 µl
DNA Template	2.0 µl
Total	25.0 µl

- Thermal Cycling

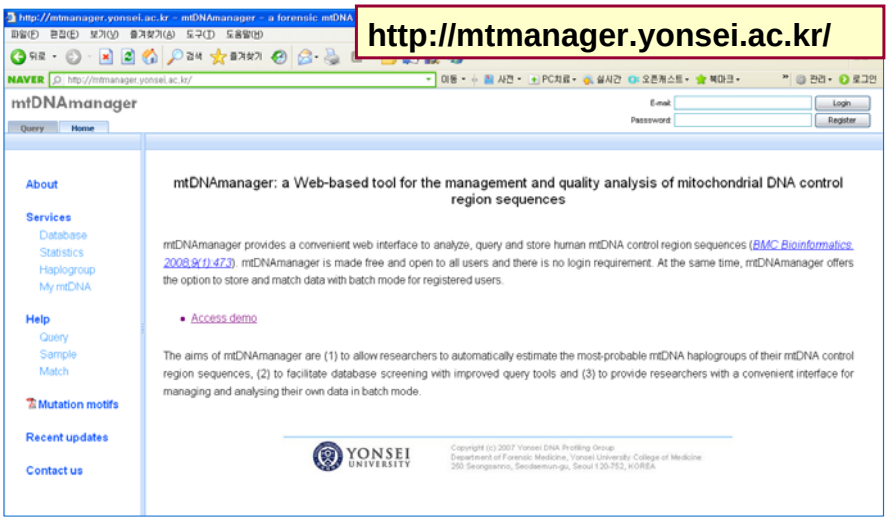
95˚C for 11 minutes , then:

95˚C for 20 seconds
50˚C for 20 seconds
72˚C for 30 seconds
for 42 cycles, then:

72˚C for 7 minutes
4˚C soak

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

Quality analysis using mtDNAmanager



YONSEI UNIVERSITY
COLLEGE OF MEDICINE

Primer for amplification of mtDNA coding region SNPs

Primer set	Haplogroup and SNP	Primer sequence (5' to 3')	Amplicon size	Concentration (μM)
PCR primer	D4-3010A	GGG ATA ACA GCG CAA TCC TA	99 bp	0.6
		ACC TTT AAT AGC GGC TGC AC		0.6
	M7-9824C	GGC ATC TAC GGC TCA ACA TT	101 bp	0.6
		ATT AGT TGG CGG ATG AAG CA		0.6
SBE primer	D4-3010A	TTT AAT AGC GGC TGC ACC AT	21 bp	0.1
	M7-9824C	GAA AGT TGA GCC AAT AAT GAC GTG	25 bp	0.1

PCR amplification of mtDNA coding region SNPs

- Monoplex PCR of mtDNA Coding Region
  - PCR Mixture

PCR component	volume
dH_20	17.0 μl
Gold ST*R 10X Buffer	2.5 μl
Forward Primer (10 pmol/μl)	1.5 μl
Reverse Primer (10 pmol/μl)	1.5 μl
AmpliTaq Gold (5 U/μl)	0.5 μl
DNA Template	2.0 μl
Total	25.0 μl

  - Thermal Cycling

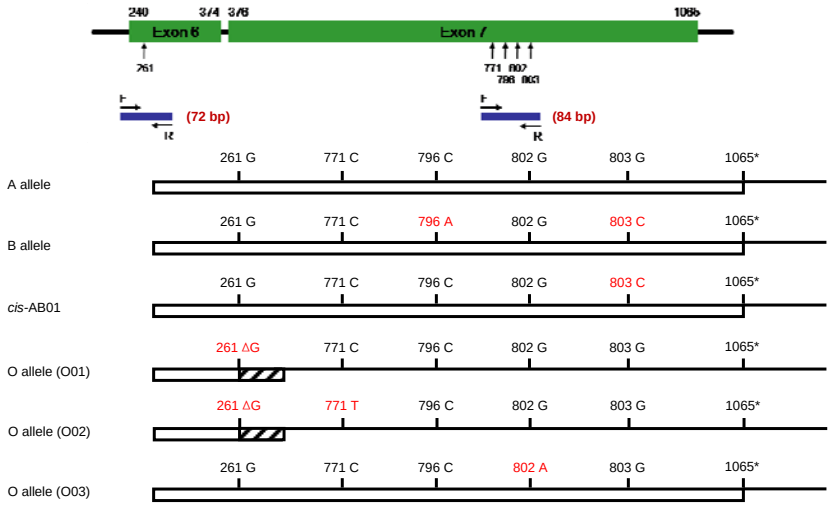
95°C for 11 minutes, then:

95°C for 20 seconds
50°C for 20 seconds
72°C for 30 seconds
for 42 cycles, then:

72°C for 7 minutes
4°C soak

- Purification of PCR Product using ExoSAP-IT
- ABI PRISM® SNaPshot® Multiplex Kit
- Purification of SNaPshot® Product using CIAP

Selection of SNPs for ABO blood group typing



Primer for amplification of ABO blood type

Primer set	Region	Primer sequence (5' to 3')	Amplicon size	Concentration (μM)
PCR primer	Exon 6	CTC CAT GTG CAG TAG GAA GGA	72 bp	0.4
		AAT GTG CCC TCC CAG ACA A		0.4
	Exon 7	CCA GTC CCA GGC CTA CAT C	84 bp	0.4
		TGC AYC TCT TGC ACC GAC		0.4
SBE primer	ABO261	AGG AAG GAT GTC CTC GTG GT	21 bp	0.23
	ABO771	$(T)_{14}$GTC CCA GGC CTA CAT CCC	33 bp	0.30
	ABO796	$(T)_{16}$GGA CGA GGG CGA TTT CTA CTA C	39 bp	0.27
	ABO802	$(T)_{24}$GGC GAT TTC TAC TAC ATG GGG	46 bp	0.30
		$(T)_{25}$GGC GAT TTC TAC TAC CTG GGG	47 bp	0.20
	ABO803	$(T)_{36}$CAC CGA CCC CCC GAA GAA C	56 bp	0.35

PCR amplification of ABO blood type

- Monoplex PCR of ABO exon 6 and 7
  - PCR Mixture

PCR component	volume
dH_20	18.0 µl
Gold ST*R 10X Buffer	2.5 µl
Forward Primer (10 pmol/µl)	1.0 µl
Reverse Primer (10 pmol/µl)	1.0 µl
AmpliTaq Gold (5 U/µL)	0.5 µl
DNA Template	2.0 µl
Total	25.0 µl

  - Thermal Cycling

95˚C for 11 minutes, then:

94˚C for 20 seconds
63˚C for 1 minute
72˚C for 30 seconds
for 45 cycles, then:

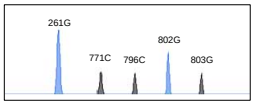
72˚C for 7 minutes
4˚C soak

- Purification of PCR Product using ExoSAP-IT
- ABI PRISM® SNaPshot® Multiplex Kit
- Purification of SNaPshot® Product using CIAP

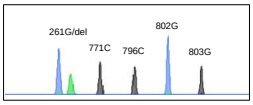
YONSEI UNIVERSITY
COLLEGE OF MEDICINE

SBE electropherograms of ABO blood type

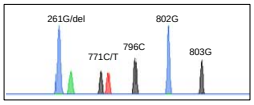
- AA



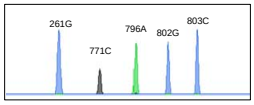
- AO01



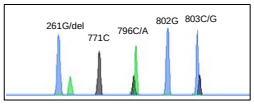
- AO02



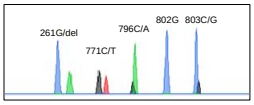
- BB



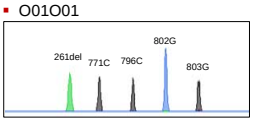
- BO01



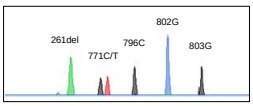
- BO02



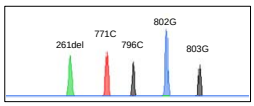
- O01O01



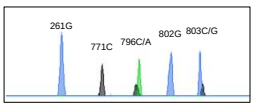
- O01O02



- O02O02



- AB

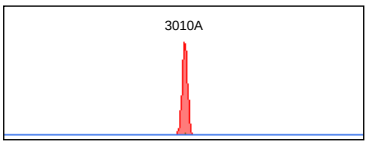


mtDNA HV region sequence and haplogroup specific SNP analysis

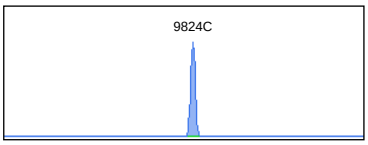
- Mummies mtDNA sequence analysis by mtDNAmanager

Mummy	Haplogroup	mtDNA hypervariable region sequence
Danwoong-mirra	D4/G	16223T-16362C-73G-263G-309.1C-315.1C-489C
Yoon-mirra	M7c1	16223T-16294T-16295T-16391A-73G-146C-199C-263G-315.1C-489C-523d-524d

- Danwoong-mirra (D4)



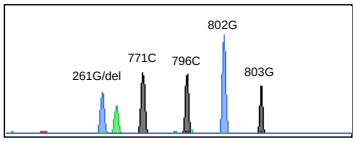
- Yoon-mirra (M7)



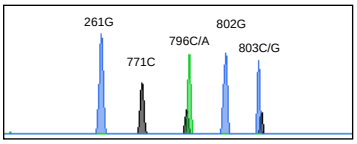
ABO blood type of Korean mummies

- Multiplex SBE reaction
- Monoplex SBE reaction
- Cloning analysis

- Danwoong-mirra (AO01)



- Yoon-mirra (AB)



Concluding remarks

- The authenticity of the results was confirmed by obtaining reproducible results from repetitive analysis of multiple extracts.
- The mtDNA haplogroup determination based on HV region sequence polymorphisms and diagnostic coding region SNP information can help confirm the absence of contamination and/or artificial recombination due to sample mix-up.
- mtDNA sequences and ABO blood types of ancient Korean mummies were successfully analyzed using a small size amplicon strategy and the authentication process, that would be used effectively in future genetic analyses of various forensic and ancient samples.

YONSEI UNIVERSITY
COLLEGE OF MEDICINE