MISSING PERSONS GENETIC IDENTIFICATION FROM COMPROMISED BONE SAMPLES

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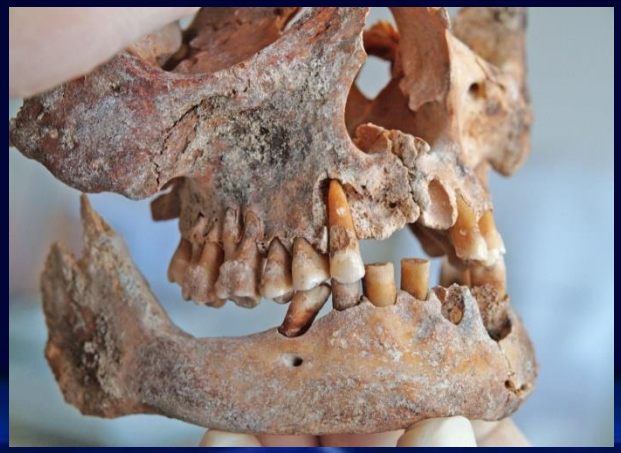


Presentation of Laboratory of Molecular Genetics at Faculty of Medicine University in Ljubljana (since 1996)

GENETIC ANALYSES

- Determination of family relationships (paternity testing, inheritance claims)
- Identification of human remains in routine forensic casework
- Identification of biological traces in criminal investigations (examination of pieces of evidence and biological traces - crime solving)
- Identification of skeletal remains of WWII victims and missing persons investigations
- Verification of identity of biological samples when the suspicion of switching of samples exsists
- Monitoring of bone marrow transplantation
- Archaeogenetic analyses of skeletal remains (molecular archaeology and historical investigation, genetic genealogy)
- Genetics of human populations

GEDNAP proficiency tests (since 1997)

- every year we participate on external proficiency tests under the leadership of GEDNAP and Stain Commission (Joint Commission of Institutes for Forensic Science and Legal Medicine)
- Autosomal STRs and amelogenin (NGM, ESI-17)
- Y-STRs (PP Y-23)
- mtDNA:
- HVI (16030-16381)
- HVII (55-388)
- Evaluation of the 3-person mixed stains in accordance with the recommendations of the Stain Commission (likelihood ratio)

Skeletal remains

- Skeletal remains - challenging biological samples for successful STR typing:

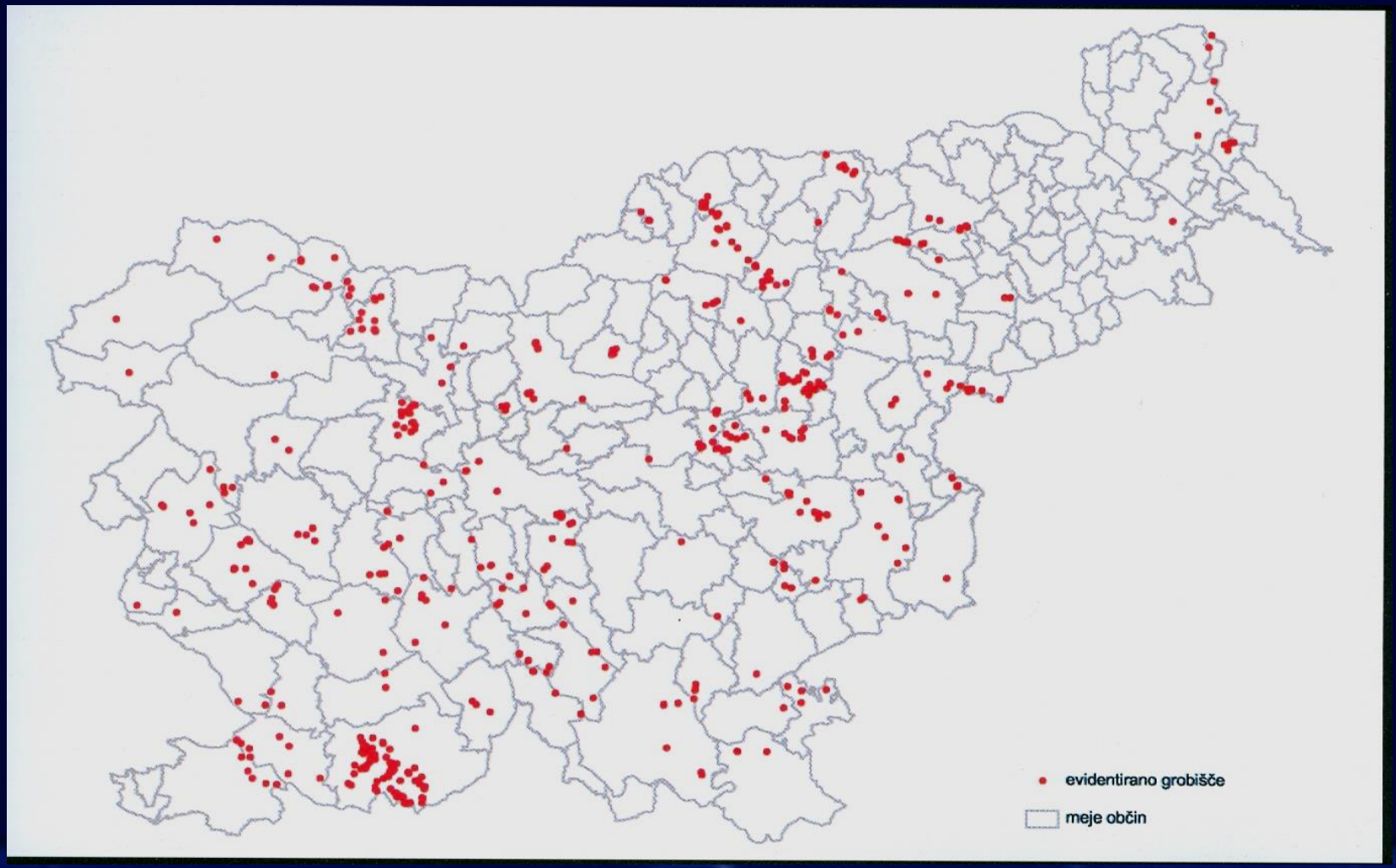
- inhibitors
- contamination with modern DNA
- degradation

Genetic typing of skeletal remains

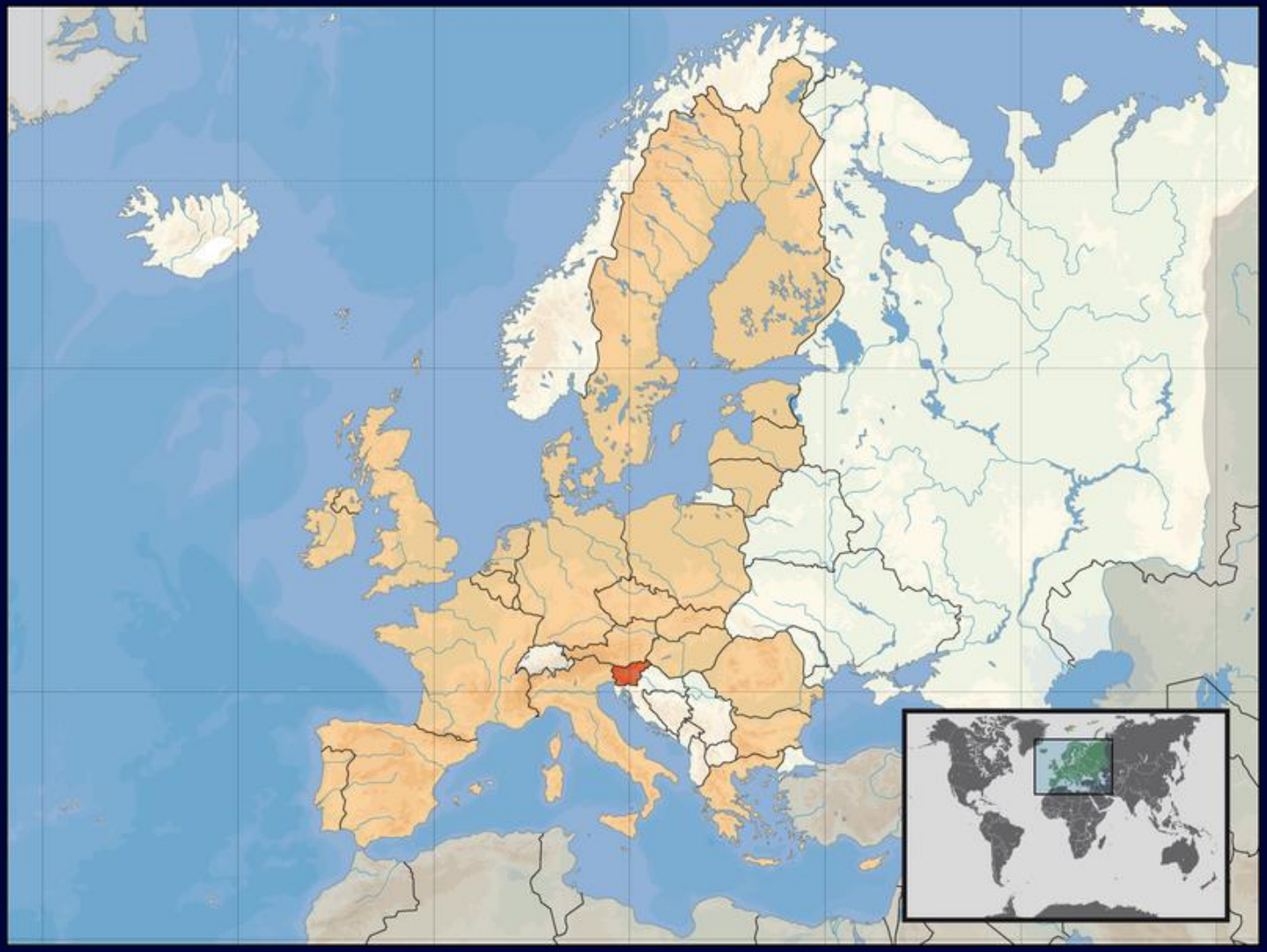
- In recent years the recovery and analysis of DNA from skeletal remains has been applied to several contexts ranging from missing person identification, disaster victim identification and identification of victims of war conflicts
- We are performing the genetic identification of skeletons from Second World War victims that have been excavated from mass graves in Slovenia and genetic analyses of skeletons from archaeological sites

WWII mass graves in Slovenia

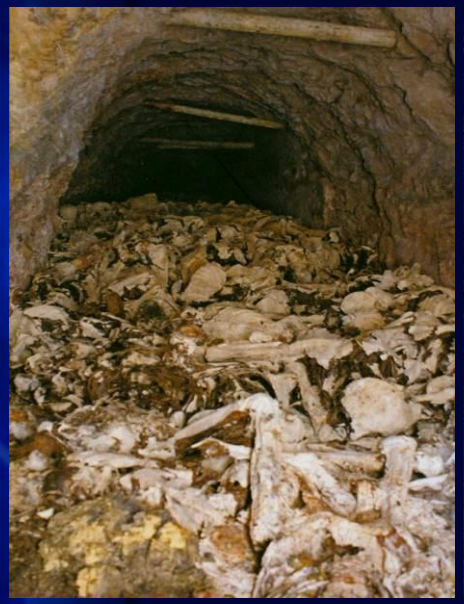
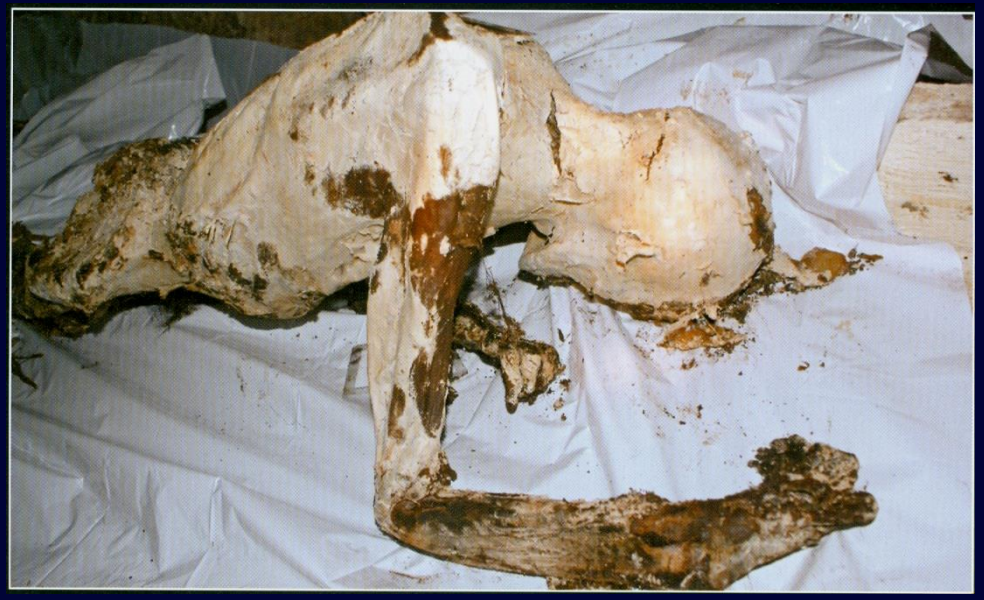
in Slovenia we have obout 600 hidden mass graves (approximatelly 100.000 victims, 15.000 Slovenians)



Slovenia – history of WWII events



Mass graves in Slovenia


8000 excavated skeletal remains:

- 4000 Germans - buried on cemetery in Ljubljana, Kranj and Celje
- Rest of them Slovenians, Croatians, ex-YU – ossuary on cemetery in Maribor

Searching for the skeletal remains

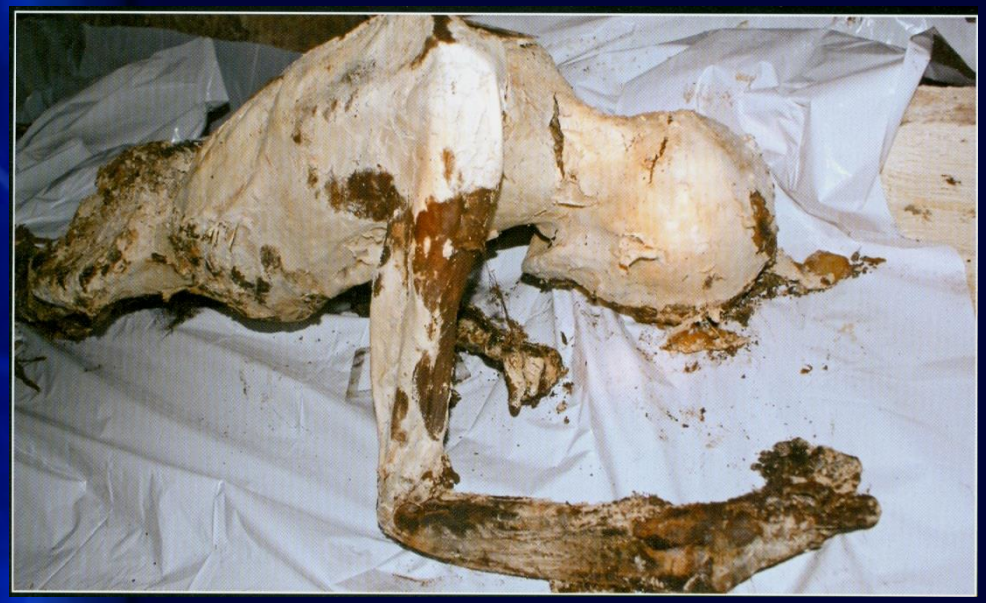
- 600 mass graves recorded by probing
- only few of them were excavated
- Probing with machinery
- Excavation performed manualy

Maribor Tezno - highway route

- Excavation of 1200 skeletons (mostly men, only 20 women, no children) on 70 m highway route
- rough estimate for the anti-tank trench (2 km 35.000 victims)
- military formation (excavated objects and military clothing)
- Croatians

Abandoned pit Huda jama near Laško

- mummified bodies in several layers

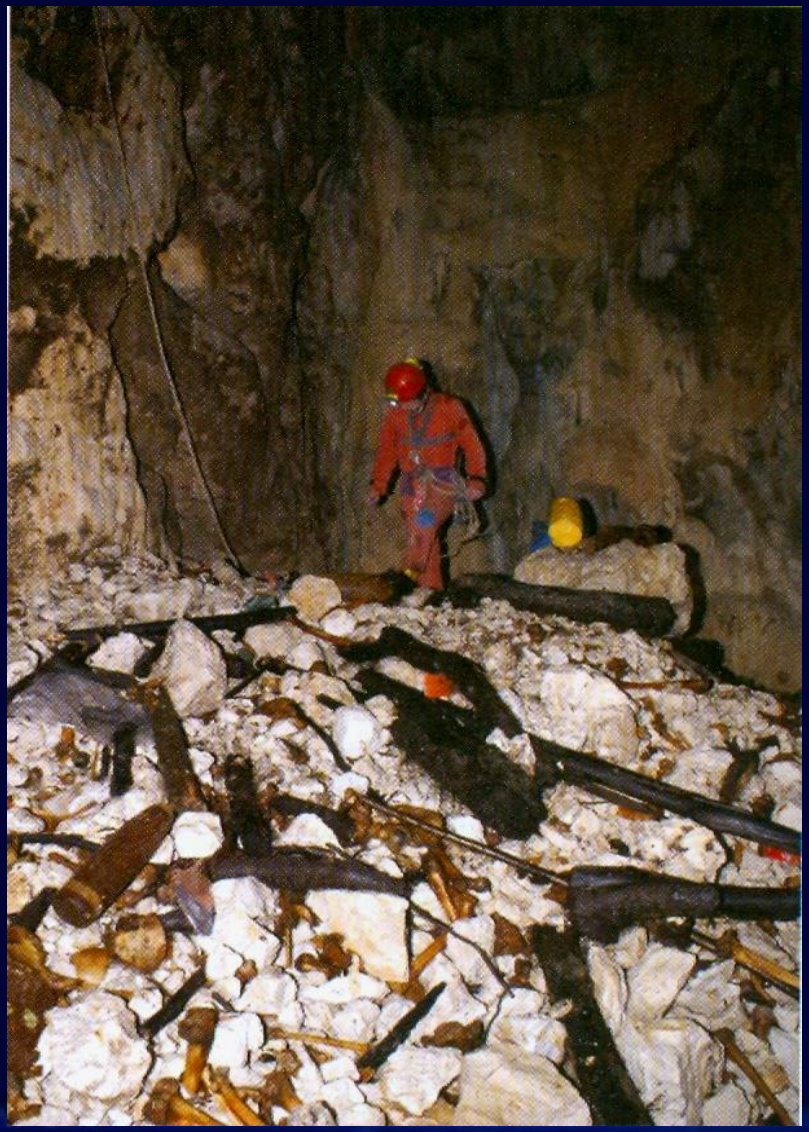


Abandoned pit Huda jama near Laško

- 1500 victims excavated
- Not only solders, also civilians

Karst Caves in Slovenia - tourist attraction

A few hundred skeletons excavated



Personal items found in mass graves

- ring hidden between the teeth

Personal items found in mass graves

- shoes, buttons, combs, scissors

Personal items found in mass graves

- leg prostheses, artificial eyes, prayers

The most common findings

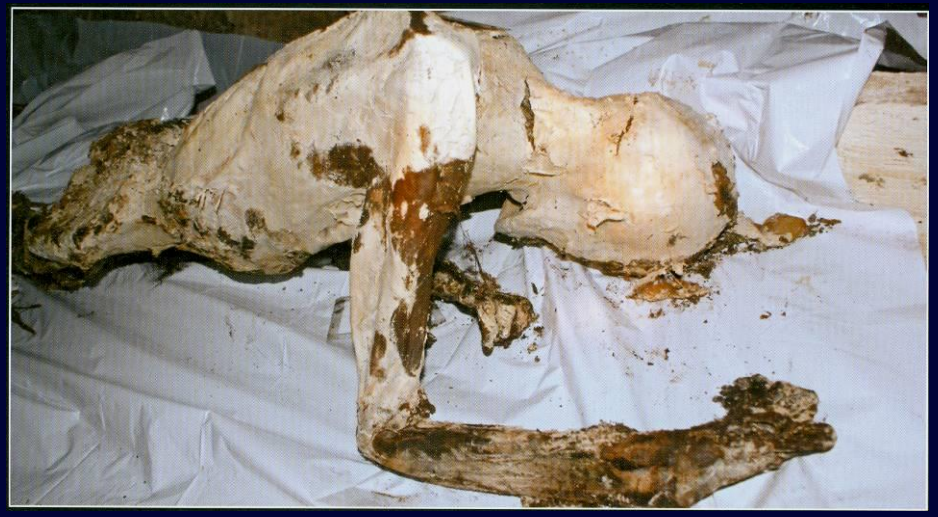
- gunshot wounds on skull
- victims were tied with wire
- mostly man victims
- military clothes (soldiers)

Professional excavation

- manually in anatomical position
- use of protective gloves, masks
- use of archaeological methods for excavation
- numbering of skeletons
- photodocumentation

Excavation in anatomical position

Whenever possible



Storage of skeletal remains

- Different parts of the body (skull with teeth) should be stored in separate paper bags to protect teeth from falling apart.
- All fragments should be marked to assure the belonging of each part to a particular skeleton.
- Regular paper is the most useful for such storing purposes.
- All skeletal remains should be photo documented.

Storage of skeletal remains

- Skeletal remains should be stored in aerial boxes. Plastic bags are not suitable, because bones can’t dry in them and the process of decay can start.
- Boxes with marked skeletal remains should be stored in dry places with low humidity to minimize the possibility for development of microorganisms.
- Sampling for genetic analyses should be performed as soon as possible.

Reccommendation for anthropologists

- The anthropologist should be informed how to handle with remains in order to prevent contamination (the use of protecting coat, mask, cap, changing gloves and sterilizing the working surface).

The nature of ancient DNA in old skeletons

- In bones and teeth binding of DNA to hydroxyapatite provides stability and preservation of DNA
- Preservation of DNA is reduced with age
- The environment surrounding the skeletal remains have the biggest effect on their preservation

The environmental factors which affect aDNA preservation

- The most significant environmental factors are:
- temperature
- humidity
- pH
- chemical characteristics of the soil
- presence of microorganisms

Temperature and humidity

- The key factors for DNA preservation are the ambient temperature and humidity (from time of death to exhumation and genetic analyses):

highly stable environments with little annual fluctuation in temperature and humidity are favorable for DNA preservation:

- caves
- permafrost

Other environmental factors that enable better preservation of DNA

- fast drying out of human remains
- minimal exposure to UV radiation
- high concentration of salt in the soil
- neutral or slightly alkaline pH of the soil
- low amount of humic acids in the soil

Storage method used after exhumation

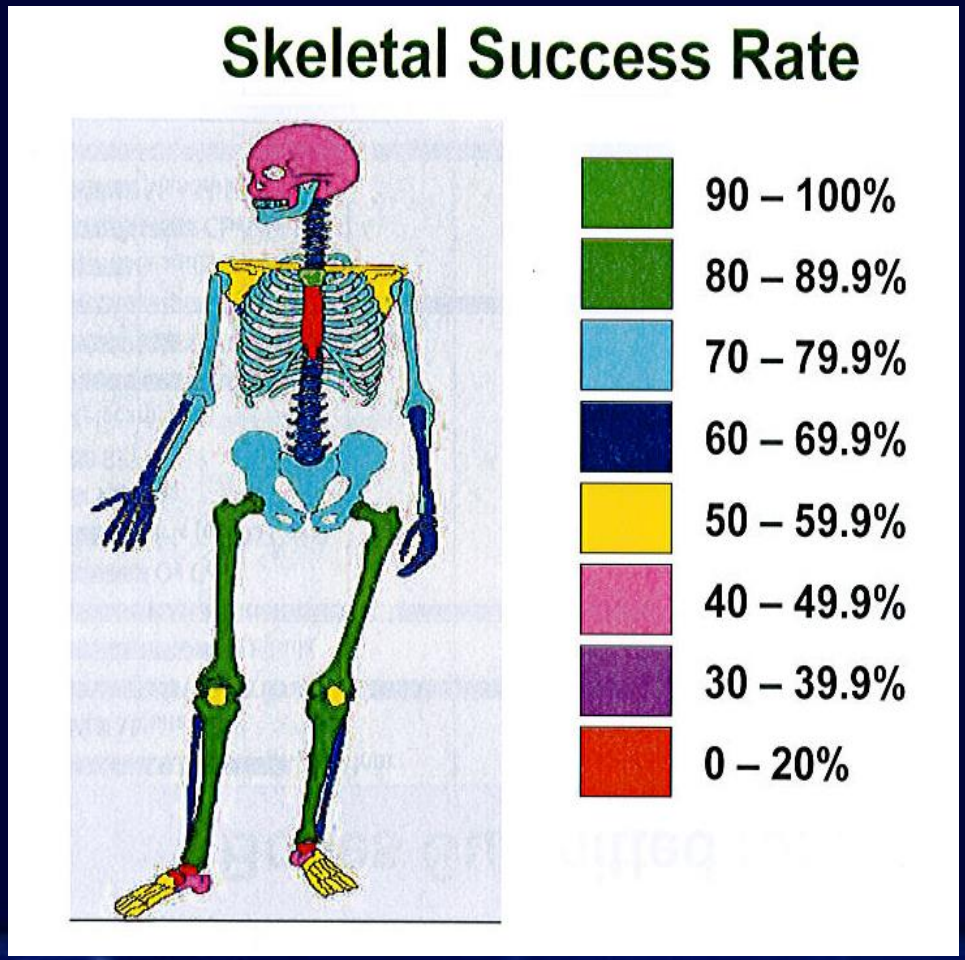
- Effectiveness of typing is much higher with freshly exhumed skeletons rather than with museum samples
- Freezing of skeletal remains is the best storage mathod
- Long-term storage: in a cool, dry, temperature-stable environment

Badly preserved skeletal remains from 17. century

The most appropriate type of bones for genetic analyses

- There is a great variation in aDNA preservation among different skeletal elements
- Long bones (femur, tibia, and humerus) are preferred over rib or other thin bones and compact (cortical) bone is preferred to spongy bone
- The skull bones are the least suitable

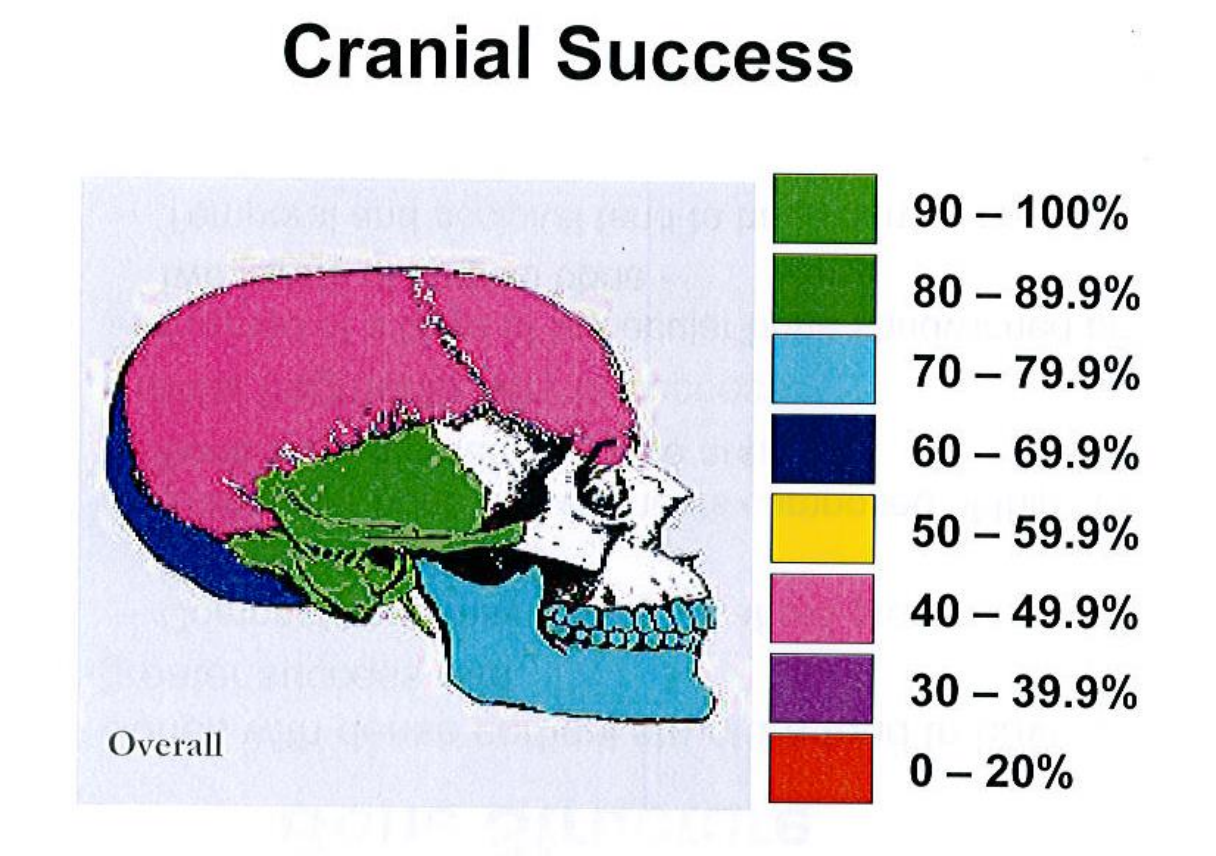
Armed Forces DNA Identification Laboratory (AFDIL)



The most appropriate type of teeth for genetic analyses

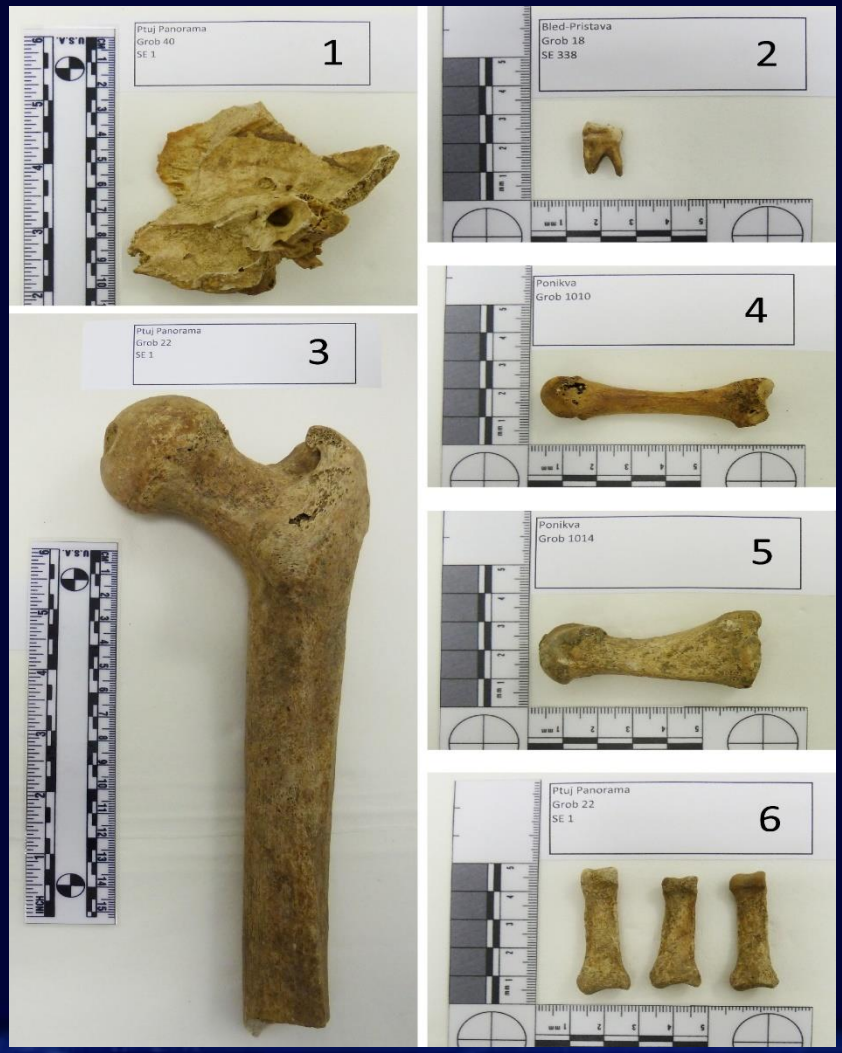
Armed Forces DNA Identification Laboratory (AFDIL)

- The amount of DNA depends on the size of the dental pulp and type of teeth; molars are the richest source of DNA, followed by, premolars, canines and incisors



Comparison of DNA content in different skeletal elements

1. temporal bone - pars petrosa
2. molar
3. femur
4. metatarsal
5. metacarpal
6. phalang bones

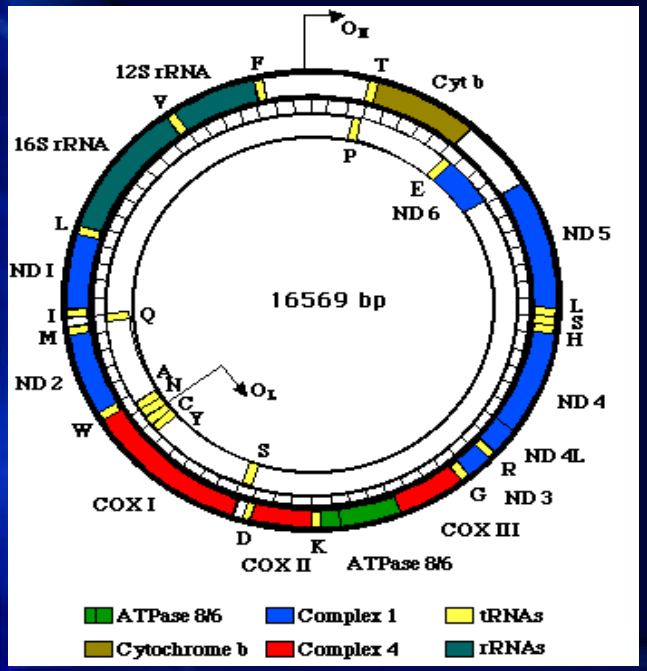


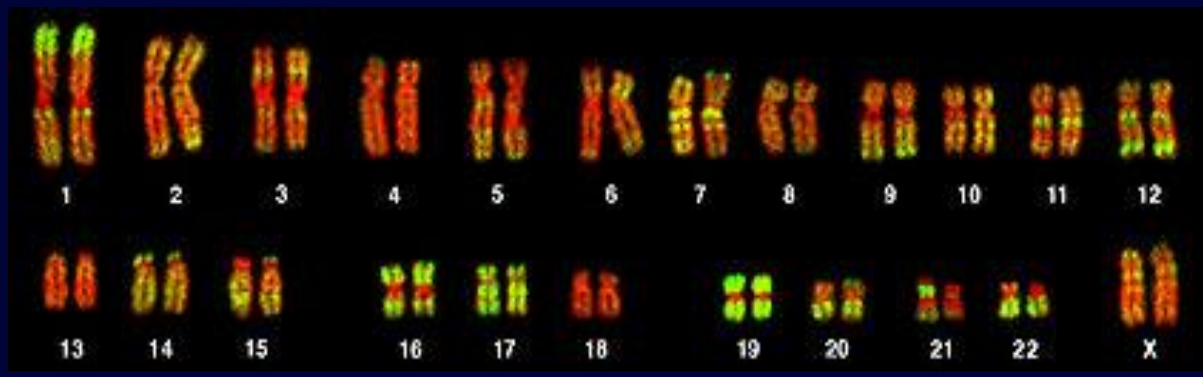
The most appropriate type of bones and teeth for genetic analyses of WWII victims

- We select for genetic testing one long bone (preferably femur) and two teeth (preferably well-preserved and endodontically untreated molars) from each individual skeleton found in the WWII mass graves; that is only possible through the excavation of skeletons in anatomic position. If not, we select for molecular genetic investigations all left or all right femurs found in the grave.

Genetic markers used for identification of WWII victims

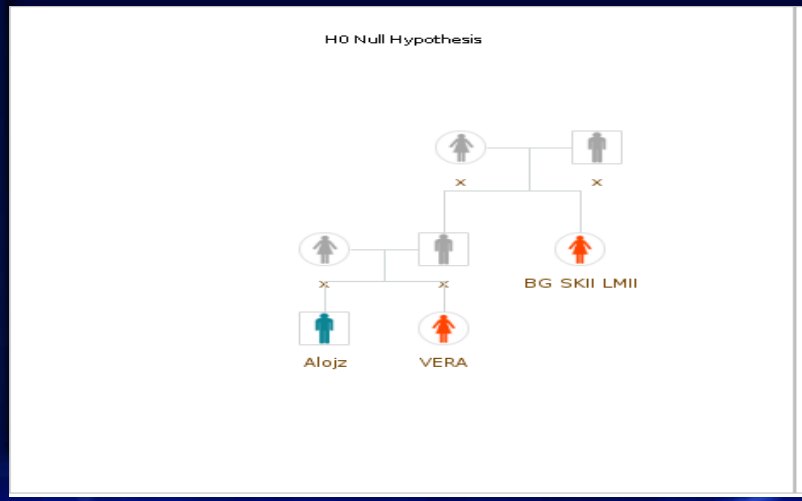
- Our experience shows that a combination of a higher number of genetic markers is neccessary for positive identification
- It is necessary to include: autosomal STRs, Y-STRs and mtDNA

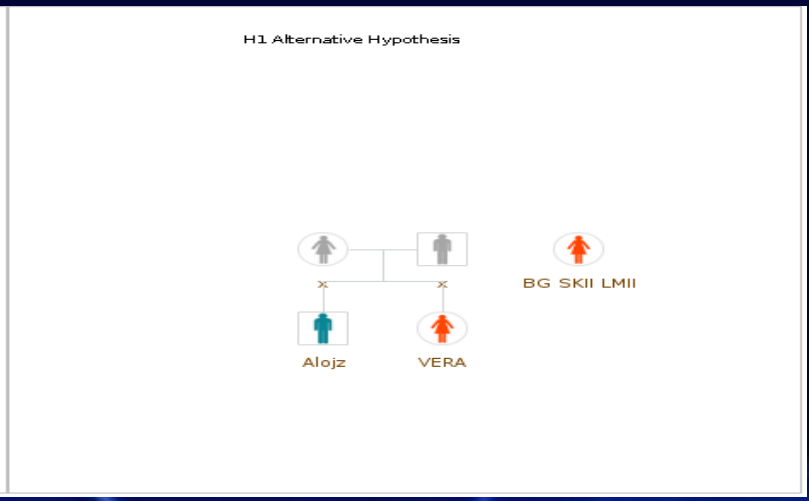




Family reference samples used for identification of WWII victims

- It is necessary to include close (brothers, sisters, sons, daughters) as well as distant (nephews, cousins) relatives of the maternal and paternal line in the identification of World War II victims to cover all genetic markers.
- Identification of aunt with living nephew and niece





Steps in DNA typing of ancient bones

- Extraction of genomic DNA
- Quantification of nuclear and mtDNA with real-time PCR
- DNA typing of autosomal and Y- STRs (PCR amplification and CE separation)
- Sequencing of HVI and HVII mtDNA
- Comparison of bone genetic profiles with negative controls and elimination database genetic profiles (to trace the contamination)
- Comparison of bone genetic profiles with reference samples from living relatives
- When match - calculation of LR and PP

Steps in DNA Analyses

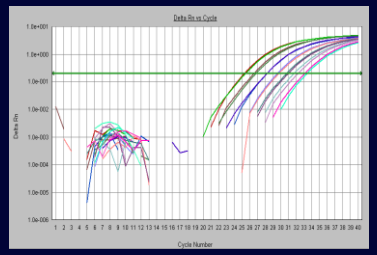
Usually 1-2 day process

Blood Stain

Buccal swab

Sample Collection & Storage

DNA Extraction



DNA Quantitation

Multiplex PCR Amplification

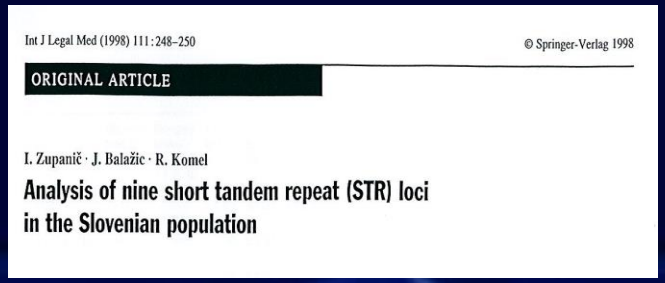
STR Typing

DNA separation and sizing

Interpretation of Results

Calculation of Match Probability

Statistics Calculated



Int J Legal Med (1998) 111:248–250 © Springer-Verlag 1998

ORIGINAL ARTICLE

I. Zupanič · J. Balažic · R. Komel

Analysis of nine short tandem repeat (STR) loci in the Slovenian population

If a match occurs, comparison of DNA profile to population allele frequencies to generate a case report with probability of a random match to an unrelated individual

Cuting, cleaning and grinding of the bones












Possibility of contamination with modern DNA

- Old and degraded samples possess very low quantity of DNA and are therefore prone to contamination with modern DNA
- Contamination can occur:
- During excavation and anthropological investigations (handling with bare hands)
- During DNA typing (laboratory persons, reagents, laboratory plastics, previously amplified PCR products)
  - ✓ An elimination database has to be performed for each mass grave to check for authenticity of genetic profiles obtained from skeletal remains

Measures for preventing DNA contamination in laboratory

- To prevent contamination with our own biological material, always use clean, sterile gloves (use double laboratory gloves) and change for every new sample. Use disposable surgical masks, caps, shoe covers and disposable laboratory coats

Measures for preventing DNA contamination in laboratory

- Laboratory working surface before and after any work undergoes regular decontamination (washing with bleach, water and ethanol). After the work the laminar flow hoods are irradiated overnight and for 30 min directly before starting to work

-Use disposable paper towels

- Clean the surface between working with different skeletal remains

Measures for preventing DNA contamination in laboratory

- Clean all tools for processing of bones and teeth after use with bleach (6% sodium hypochlorite) or with DNA Away

- Wash away the detergent with several washes with water and ethanol and leave tools to air dry

Measures for preventing DNA contamination in laboratory

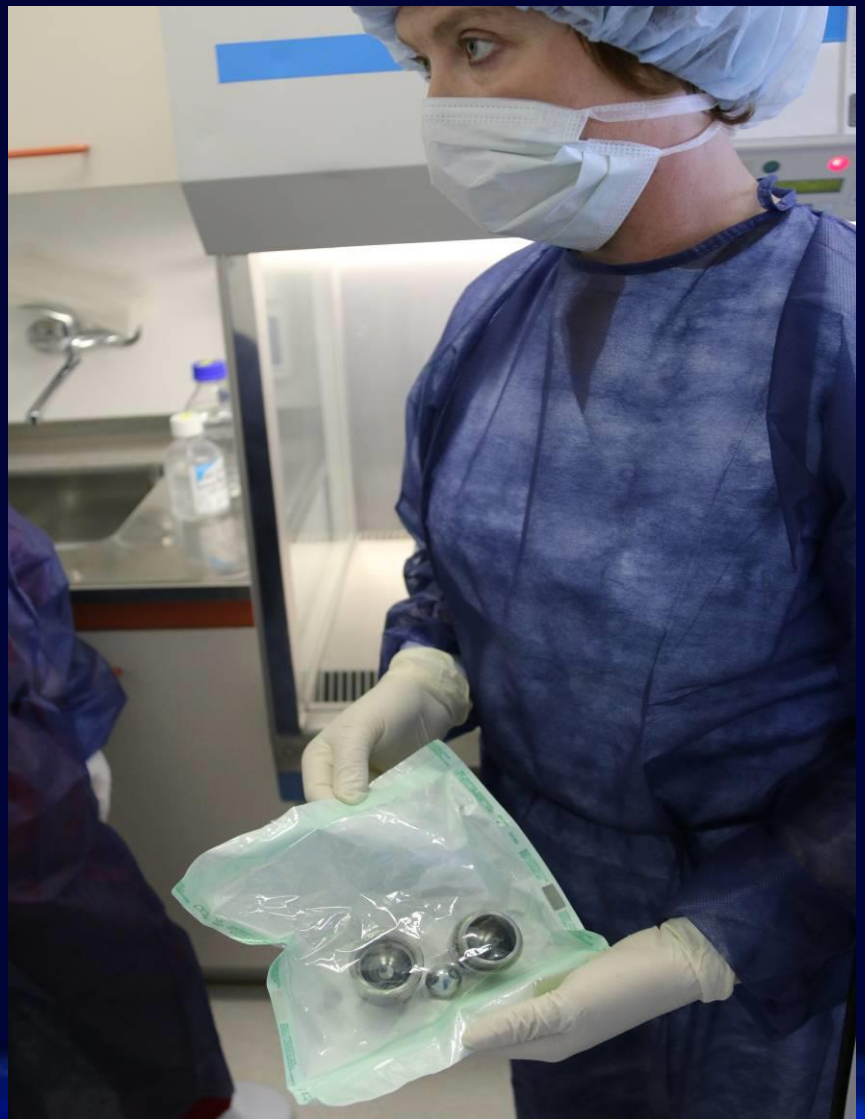
- Tools are cleaned and stored in plastic bags, sterilized and UV irradiated at least overnight or up to 72 hours and for 30 min directly before starting to work

Measures for preventing DNA contamination in laboratory

- Put all the reagents, tools and laboratory plastics under UV light before starting to work

Measures for preventing DNA contamination in laboratory

- To avoid cross-contamination among samples a different set of equipment is used for each sample (such as grinding jar, cutting saw blades, drilling bits, tweezers, forceps and spatulas)

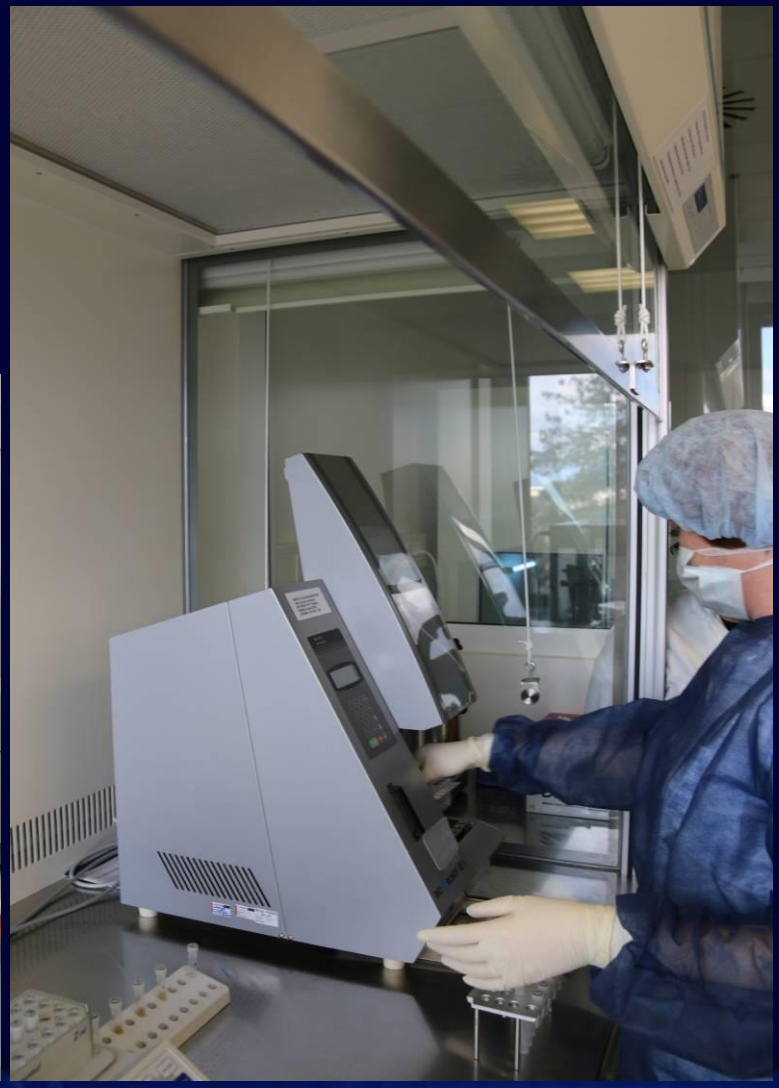


Measures for preventing DNA contamination in laboratory

- Analyse bone and teeth samples separately from reference and elimination database samples
- We use physically separated room for processing bone and tooth samples. Analyses of skeletons should be also temporally separated from reference and elimination database samples

Measures for preventing DNA contamination in laboratory

- The separation of pre- and post-PCR procedures must be provided to prevent contamination with previously amplified products



Measures for preventing DNA contamination in laboratory

- We have different rooms in pre-PCR laboratory to separate each step in the bone typing procedure

- In each room we have laminar flow hoods with shortwave (254 nm) UV source and hepa-filters.

Measures for preventing DNA contamination in laboratory

- In room for cleaning and grinding the bones and teeth we clean the bones mechanically in a closed microbiological safety cabinet to capture and remove the bone powder that is released into the air during drilling and cutting. It has strong airflow to the filters that collect the dust at the bottom of the chamber

It is necessary to separate the dust-producing working steps from the contamination-susceptible steps like buffer preparation and PCR setup. The laboratory set-up must prevent dust from contaminating the rest of the process in DNA typing of skeletal remains.

Measures for preventing DNA contamination in laboratory

- The extraction room is used for decalcification, extraction and purification

Measures for preventing DNA contamination in laboratory

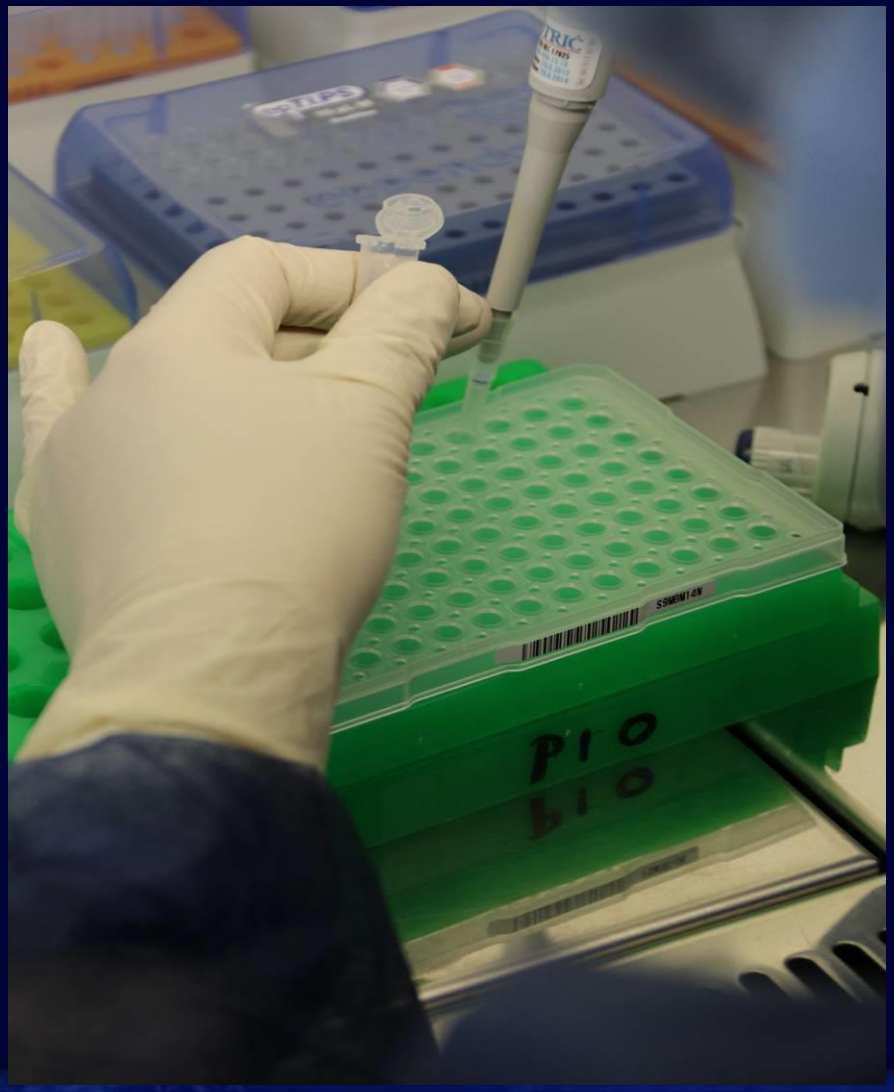
- The PCR room is used for setup of PCR reagent mix (first hood) and addition of DNA extracts to the PCR (second hood)

Measures for preventing DNA contamination in laboratory

- For monitoring the cleanliness of the isolation reagents and laboratory plastics, and cross-contamination during the procedure we always use extraction negative control

Measures for preventing DNA contamination in laboratory

- To detect any possible contamination with DNA or previously amplified PCR products of reagents or laboratory plastics, we always use negative control in the PCR



Measures for preventing DNA contamination in laboratory

- All genetic profiles obtained from skeletal remains are compared to elimination database

Vzorec	D8S1179		D21S11		D7S820		CSF1PO		D3S1358		THO1		D13S317		D16S539	
P. L. S.	14	14	30.2	30.2	10	12	11	12	15	17	8	9	12	13	8	12
I. Z. P.	13	15	30	32.2	9	10	9	11	14	18	6	9	11	11	11	12
B.G.P.	10	14	29	31	10	11	12	12	14	17	7	9.3	8	11	9	9
K. V.	13	16	28	30	8	10	10	11	16	16	8	9	11	13	9	12
K. I.	12	13	30	31.2	8	8	12	13	18	18	8	9.3	9	11	12	12
G. M.	12	14	28	29	9	12	11	12	14	15	6	6	11	12	12	12
R. B.	13	14	30	32.2	10	11	9	12	15	16	6	9.3	10	12	10	12
An. M.	10	12	28	30	8	10	9	11	15	17	9	9	12	13	12	12
D.J.	13	15	28	29	8	10	10	11	14	15	9.3	9.3	11	12	11	13
P.P.	13	14	28	29	9	9	11	11	14	15	6	9.3	12	12	12	12
A.S.S.	10	13	29	30	10	11	11	12	15	17	6	9	11	12	10	11
P.R.	15	15	30	30	8	13	11	13	16	17	8	9.3	11	11	11	12
D.H.	10	11	29	29	8	11	10	11	17	19	6	9.3	12	12	8	12
Al.M.	11	15	30	32.2	10	12	11	12	15	15	6	9	8	11	11	12
P.J.	12	12	28	28	11	11	9	12	16	18	6	9.3	11	12	9	12
Vzorec	D2S1338		D19S433		vWA		TPOX		D18S51		Amelog.		D5S818		FGA	
P. L. S.	17	25	14	14	16	16	11	11	15	18	X	X	12	13	21	24
I. Z. P.	24	26	15	16	17	18	8	11	12	16	X	X	12	12	22	24
B. G. P.	17	20	13	15.2	14	16	8	12	10	17	X	X	11	11	20	20
K. V.	17	25	13	14	18	18	8	11	17	19	X	X	11	13	19	20
K. I.	20	24	13	13	14	17	8	10	14	17	X	X	13	14	22	24
G. M.	17	17	14	16	17	17	8	9	12	18	X	Y	12	13	20	21
R. B.	17	21	14	14	14	19	8	9	14	19	X	Y	11	12	21	24
An. M.	17	25	13	14	16	17	8	11	12	15	X	Y	12	12	24	25
D.J.	17	19	14.2	16	14	16	8	9	16	19	X	Y	11	12	21	23
P.P.	18	24	14	14	16	17	8	9	12	15	X	Y	12	13	18	25
A.S.S.	18	20	14	14	17	17	11	11	17	21	X	Y	10	12	19	22
P.R.	18	20	13	13.2	17	18	8	8	12	17	X	Y	12	12	19	25
D.H.	17	20	14	16	18	19	11	11	13	16	X	Y	11	12	22	24
Al.M.	17	19	15	15.2	18	18	11	12	15	18	X	Y	11	11	21	23.2
P.J.	20	23	13	15	17	18	8	8	13	18	X	Y	11	12	21	23

Vzorec	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385a/b	DYS393
G. M.	15	14	24	31	17	16	14/15	13
R. B.	15	13	24	30	16	17	14/15	13
An. M.	17	13	25	30	15	16	11/14	13
D.J.	17	13	25	30	16	13	16/18	13
P.P.	15	12	22	28	15	14	13/15	13
A.S.S.	15	13	25	29	17	16	14/15	13
P.R.	17	14	25	31	14	16	11/14	13
D.H.	16	13	25	31	15	15	11/14	13
Al.M.	17	13	25	30	16	16	11/14	13
P.J.	15	13	24	31	18	15	14/15	13
Vzorec	DYS391	DYS439	DYS635	DYS392	DYSH4	DYS437	DYS438	DYS448
G. M.	11	13	23	11	11	15	10	20
R. B.	11	13	23	11	11	15	10	20
An. M.	10	10	23	11	12	14	11	20
D.J.	10	11	23	11	12	14	10	20
P.P.	10	11	21	11	10	16	10	20
A.S.S.	11	13	23	11	11	15	10	20
P.R.	11	10	23	11	12	14	11	20
D.H.	10	12	23	11	11	14	11	20
Al.M.	10	10	23	11	12	14	11	20
P.J.	11	13	23	11	11	15	10	20

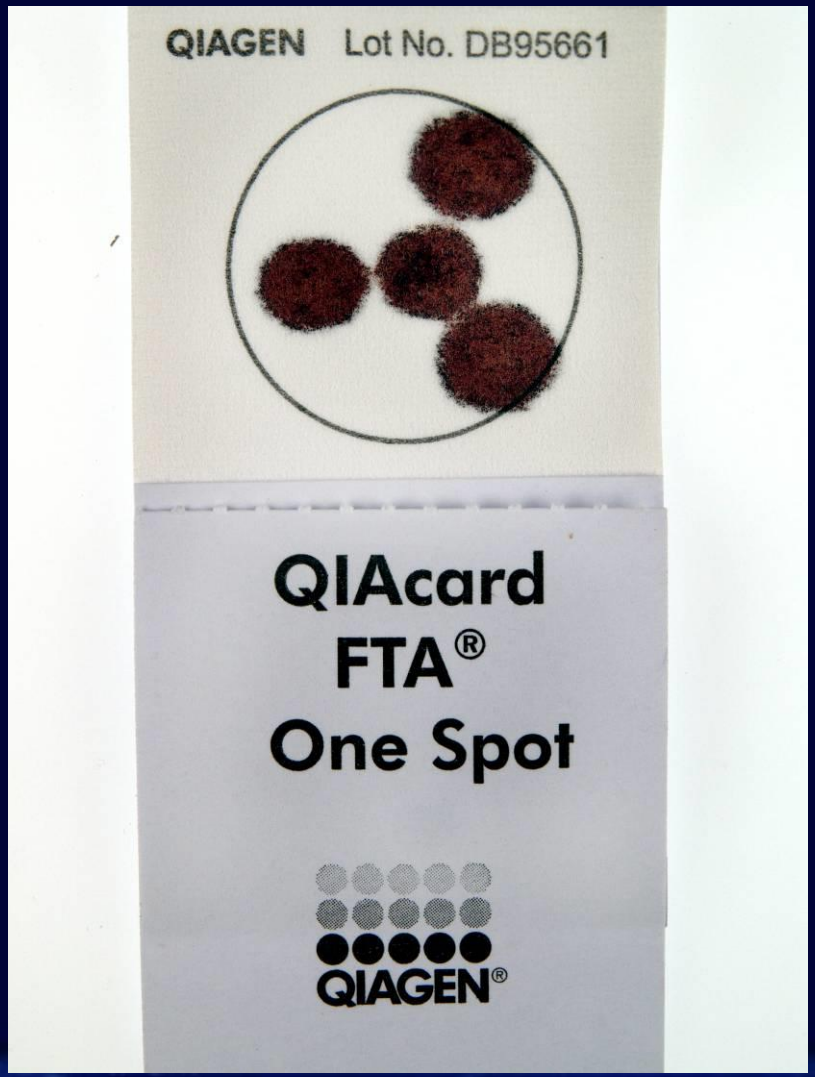
Vzorec	Razlike glede na "CRS"	Območje
P. L. S.	HVI: 16298C HVII: 72C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407
I. Z. P.	HVI: 16343G HVII: 73G, 150T, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
B. G. P.	HVI: 16126C, 16182C, 16183C, 16189C, 16294T, 16296T, 16298C, 16357C HVII: 73G, 195C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
K. V.	HVI: 16298C HVII: 72C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
K. I.	HVI: 16311C, 16362C HVII: 239C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
G. M.	HVI: 16362C HVII: 239C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
R. B.	HVI: identična CRS HVII: 152C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407
An. M.	HVI: 16069T, 16126C HVII: 73G, 185A, 188G, 228A, 263G, 295T, 315.1C	HVI: 16030-16400 HVII: 55-407
D.J.	HVI: 16261T HVII: 200G, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
P.P.	HVI: 16126C, 16294T, 16296T, 16304C HVII: 73G, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
A.S.S.	HVI: 16298C HVII: 72C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
P.R.	HVI: 16362C, 16400T HVII: 239C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
D.H.	HVI: 16126C, 16292T, 16294T, 16296T, 16304C HVII: 73G, 263G, 309.1C, 315.1C, 321C	HVI: 16030-16400 HVII: 55-407
Al.M.	HVI: 16126C, 16294T, 16296T, 16304C HVII: 73G, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
P.J.	HVI: 16170G, 16390A HVII: 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407

Measures for preventing DNA contamination in laboratory

- Always use filter tips to minimize the risk of aerosol contamination. Tips are exposed to UV light before use

Measures for preventing DNA contamination in laboratory

- We use the room for processing old bones and teeth exclusively for this kind of biological material and not for high-template DNA samples (saliva, blood)



Measures for preventing DNA contamination in laboratory

We isolate DNA from bones and teeth at least twice (from a different skeletal element of the same individual when possible) to check the results of genotyping and for interpretation reproducible results are used

The method of DNA extraction from bones and teeth

- It was developed in our laboratory to acquire high quality DNA from WWII skeletons and skeletons from archaeological site (contemporary skeletal remains)

Journal of Forensic and Legal Medicine 37 (2016) 78–86

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

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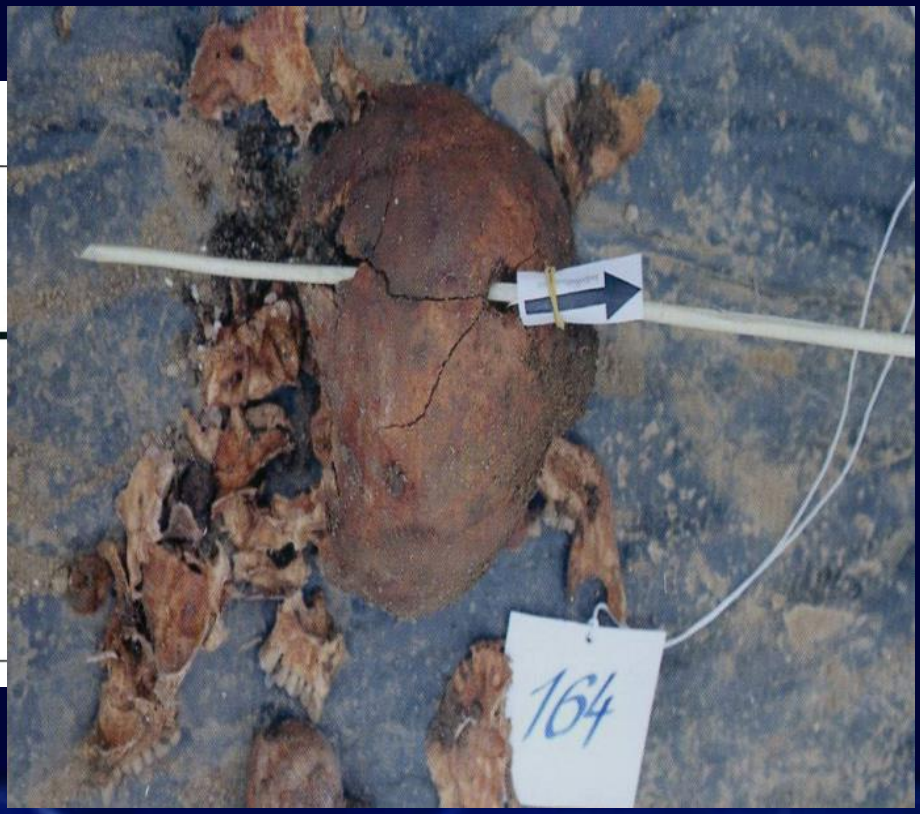
Irena Zupanič Pajnič [a, *, 1], Magdalena Debska [b, 1], Barbara Gornjak Pogorelc [a], Katja Vodopivec Mohorčič [a], Jože Balažic [a], Tomaž Zupanc [a], Borut Štefanič [a], Ksenija Geršak [c]

[a] Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia
[b] Central Forensic Laboratory of the Police, Research Institute, Aleje Ujazdowskie 7, 00-583 Warsaw, Poland
[c] Division of Medical Genetics, Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šlajmerjeva 2, Ljubljana, Slovenia



The method of DNA extraction from bones and teeth

- To evaluate this method we analysed more than 150 WWII bones and bones from archaeological sites

Zdrav Vestn | marec 2011 | Letnik 80 IZVIRNI ČLANEK/ORIGINAL ARTICLE

Visoko učinkovita metoda ekstrakcije DNA iz skeletnih ostankov

Highly efficient DNA extraction method from skeletal remains

Irena Zupanič Pajnič

Ancient skeletons from archaeological sites

IZVIRNI ČLANEK/ORIGINAL ARTICLE

Molekularnogenetska preiskava 300 let starih skeletov iz Auerspergove grobnice

Molecular genetic analyses of 300-year old skeletons from Auersperg tomb

Irena Zupanič Pajnič

The method of DNA extraction from bones and teeth

- Our protocol was established on total demineralization process using EDTA (enables separation of bone cells from the bone mass and demineralization of bone matrix)
- Total demineralization significantly increases the proportion of full profiles, reflecting a correlation with better DNA quality

Forensic Sci Int Genet. 2007 Jun;1(2):191-5. doi: 10.1016/j.fsigen.2007.02.006. Epub 2007 Mar 12.

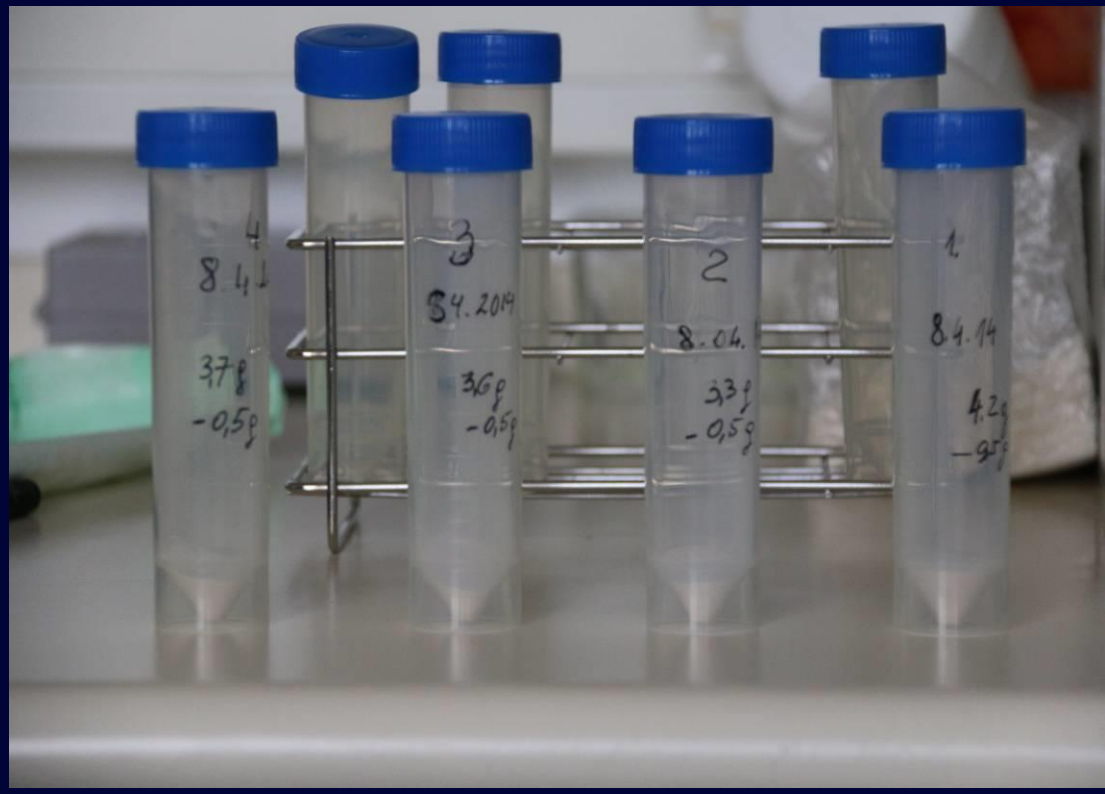
ELSEVIER FULL-TEXT ARTICLE

High efficiency DNA extraction from bone by total demineralization.

Loreille OM[1], Diegoli TM, Irwin JA, Coble MD, Parsons TJ.

The method of DNA extraction from bones and teeth

- It proved effective from relatively small 0.5-g bone or tooth powder
- It avoids overly aggressive treatments (high temperature or use of strong detergents) to reduce further degradation of already damaged aDNA



Extraction procedure

1. Cleaning of the bones for remove surface contamination and inhibitors:

   - Mechanical cleaning (physical removal of bone surface with drilling; in tooth samples radiation with UV). To prevent bone warming during drilling and cutting we frequently use liquid nitrogen

   - Chemical cleaning (washing in detergent, water and ethanol)
2. Powdering of the bones
3. Decalcification and lysis
4. Purification of genomic DNA

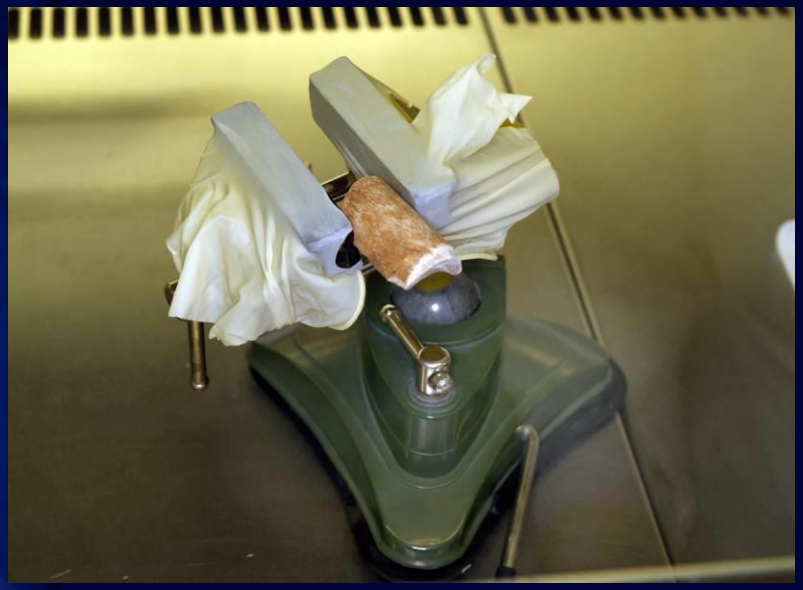
Cleaning of the bones

- Femur (cut 8-10 cm below trochanter)
- Mechanical cleaning - close cytostatic safety cabinet (in separated room)

Cleaning of the bones

- removing of dirt, soil mechanicaly
- cleaning in water and detergent
- washing in water several times
- overnight drying

Drilling of the bones



Mechanical cleaning - physical removal of bone surface (1-3 mm) using a rotary sanding tool:

-fasten bone into holding vice

-use liquid nitrogen

Chemical cleaning of the bones

- Washing with 5% Alconox detergent, sterile bi-distilled water and 80 % ethanol
- overnight drying

Bone powdering

- use of liquid nitrogen to avoid overheating during powdering
- metal jar and metal ball

Bone powdering

- MillMix (Domel)
- 1-2 min. , frequency of 30 Hz
- Very fine powder
- For extraction 0.5 g

Decalcification and lysis

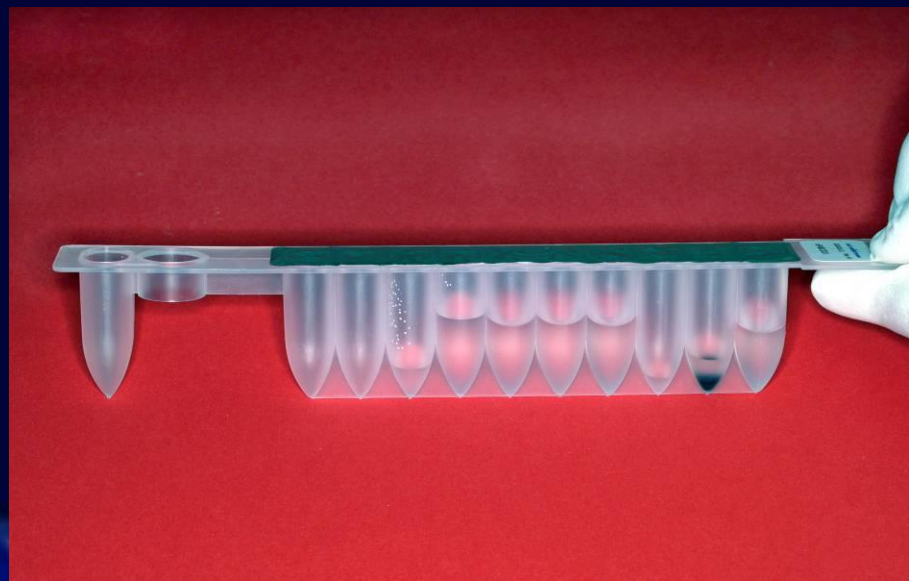
- decalcification with EDTA at 37 C overnight (for total demineralization 15 ml of 0.5 M EDTA is needed for 1 g)
- lysis with proteinase K, DTT and extraction buffer at 56 C for 2 hours
- extraction negative control

Purification of genomic DNA

- purification in a Biorobot EZ1 (EZ1 DNA Investigator Kit - Qiagen)
- automated (20 min)
- based on technology of magnetic particles covered with silicon (efficient for binding DNA)
- no use of toxic organic solvents (phenol, chloroform)

Purification of genomic DNA

- purification process is done in a huge filter tip
- purification reagents are placed in cartridge
- all plastics and reagents for single use only
- no manual pipetting
- important for prevention of contamination
- purification using magnetic particles (also other robotic machines – AutoMate Express, Maxwell, manually)



AutoMate Express

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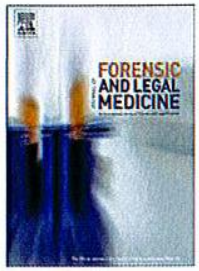
journal homepage: www.elsevier.com/locate/jflm



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[a] Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia
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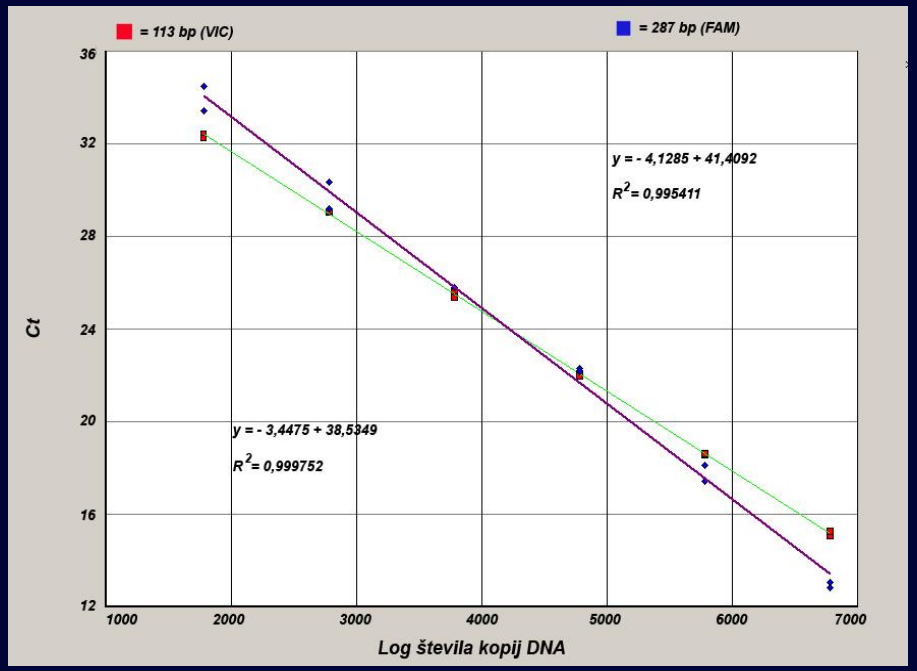
[c] Division of Medical Genetics, Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šlajmerjeva 2, Ljubljana, Slovenia

Quantification of nuclear and mtDNA

- The nuclear and mtDNA of bone and tooth samples are quantified in our laboratory using real-time PCR
- mtDNA in-house assay (100, 300 bp - degradation)
- Nuclear DNA - Human Quantifiler and PowerQuant (degradation index)

Quantification of mtDNA



ELSEVIER
FULL-TEXT ARTICLE

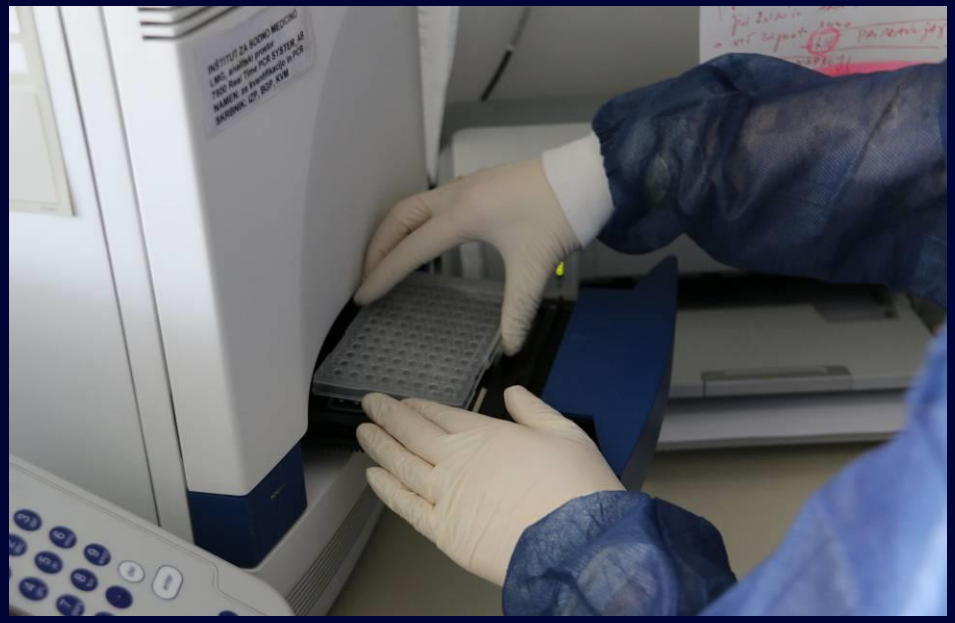
Forensic Sci Int. 2004 Jan 28;139(2-3):141-9.

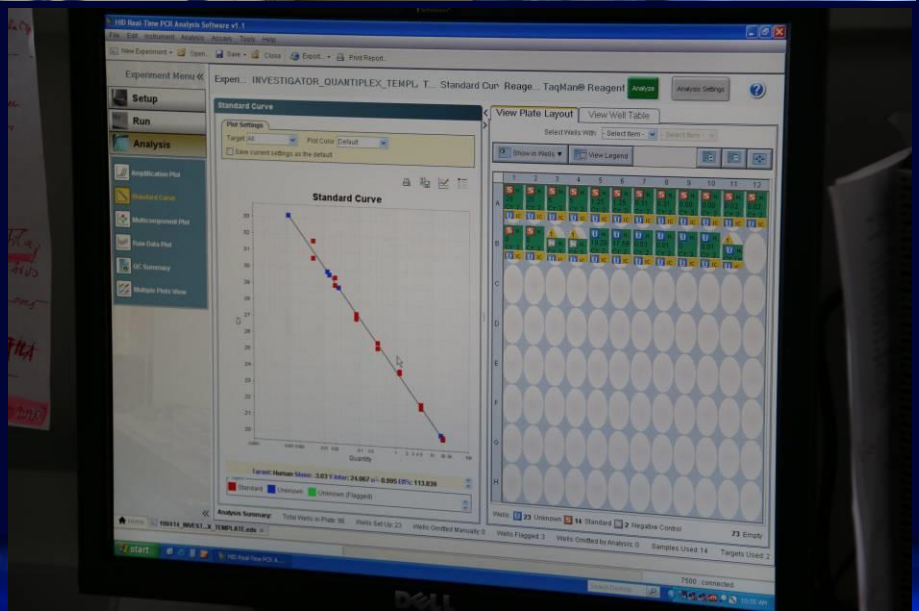
Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies.

Alonso A[1], Martín P, Albarrán C, García P, García O, de Simón LF, García-Hirschfeld J, Sancho M, de La Rúa C, Fernández-Piqueras J.

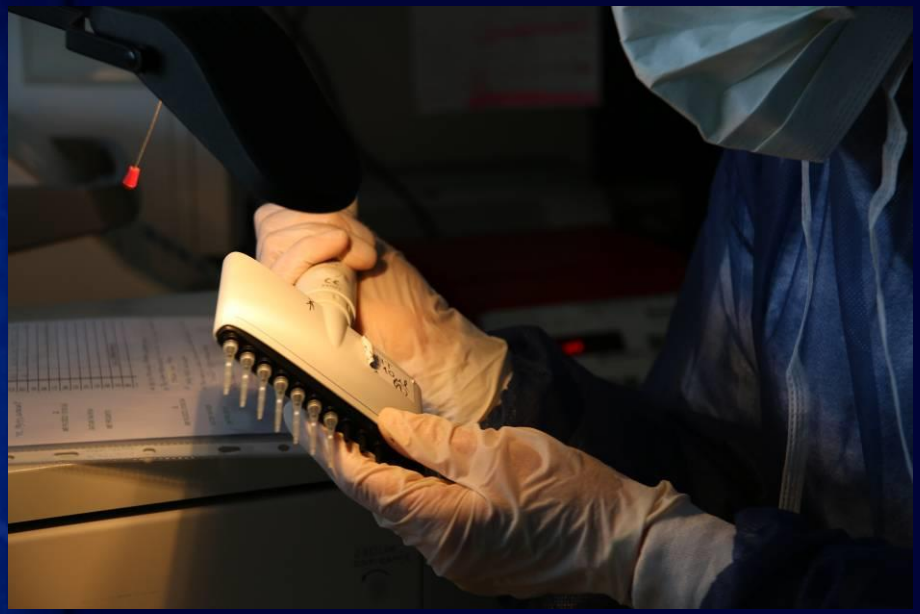
Quantification of nuclear DNA

- Estimation of presence of inhibitors
- extracts with higher amount of nuclear DNA are used for STR typing (autosomal and Y-STRs)
- extracts with lower amount of nuclear DNA are used for mtDNA typing





PCR amplification and separation on CE



Performance of different ampification kits- which kit to use for autosomal STR typing?

- When testing the performance of NGM, ESSPlex and ESX 17 using bone extraction method optimised in our laboratory DNA typing of WWII skeletal remains was successful in 96 % of the samples with all of them and very few allelic drop-outs were observed

FORENSIC SCIENCE

CMJ 17

doi: 10.3325/cmj.2012.53.17

Highly efficient nuclear DNA typing of the World War II skeletal remains using three new autosomal short tandem repeat amplification kits with the extended European Standard Set of loci

Irena Zupanič Pajnič, Barbara Gornjak Pogorelc, Jože Balažic, Tomaž Zupanc, Borut Štefanič

Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana

Performance of different ampification kits

Rom J Leg Med [21] 73-78 [2013]
DOI: 10.4323/rjlm.2013.73

A Comparative Analysis of the AmpFlSTR Identifiler and PowerPlex 16 Autosomal Short Tandem Repeat (STR) Amplification Kits on the Skeletal Remains Excavated from Second World War Mass Graves in Slovenia

*Irena Zupanič Pajnič**

Rom J Leg Med [21] 119-124 [2013]
DOI: 10.4323/rjlm.2013.119

Performance of the Human Quantifiler, the Investigator Quantiplex and the Investigator ESSplex Plus kit for quantification and nuclear DNA typing of old skeletal remains

*Tomaz Zupanc, Joze Balazic, Borut Stefanic, Irena Zupanic Pajnic**

Forensic Science International: Genetics 27 (2017) 17–26

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journal homepage: www.elsevier.com/locate/fsig

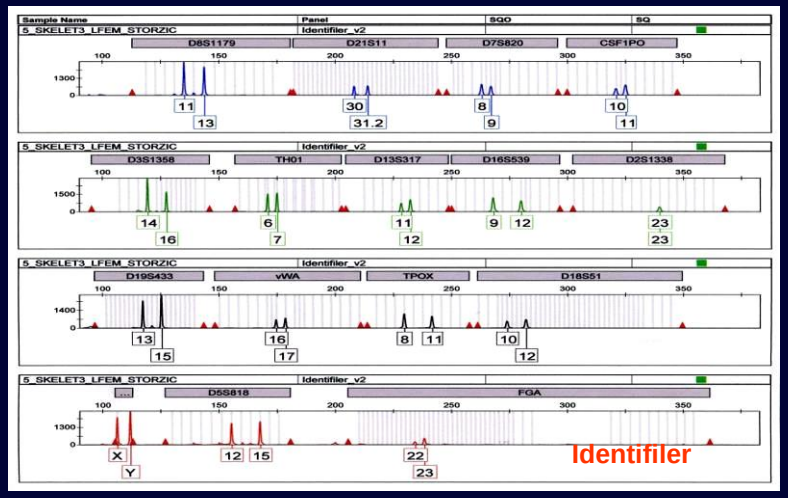
Research paper

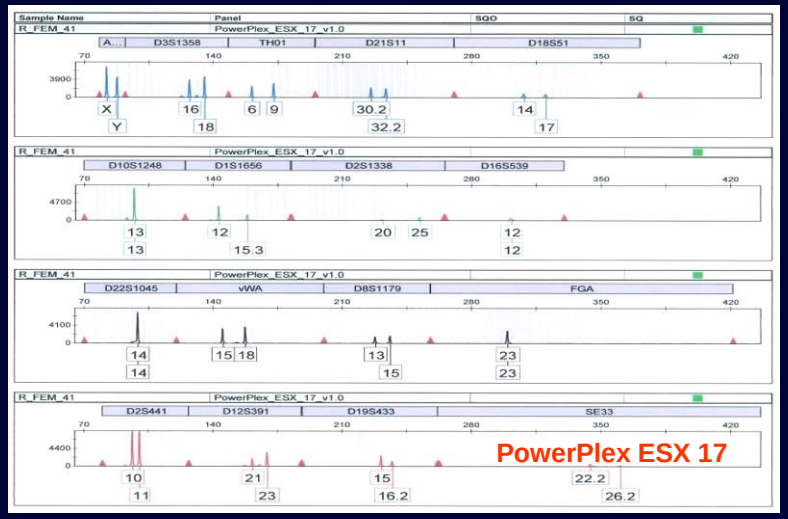
Prediction of autosomal STR typing success in ancient and Second World War bone samples

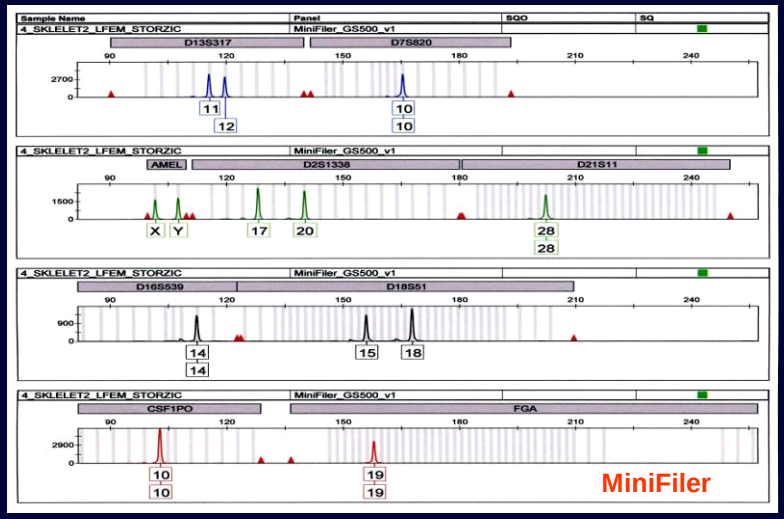
Irena Zupanič Pajnič[a,*], Tomaž Zupanc[a], Jože Balažic[a], Živa Miriam Geršak[a], Oliver Stojković[b], Ivan Skadrić[b], Matija Črešnar[c,d]

[a] Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia
[b] Institute for Legal Medicine, Faculty of Medicine, University of Belgrade, Deligradska 31, 11000 Belgrade, Serbia
[c] University of Ljubljana, Faculty of Arts, Department of Archaeology, Aškerčeva 2, 1000 Ljubljana, Slovenia
[d] Institute for the Protection of Cultural Heritage, Centre for Preventive Archaeology, Poljanska 40, 1000 Ljubljana, Slovenia

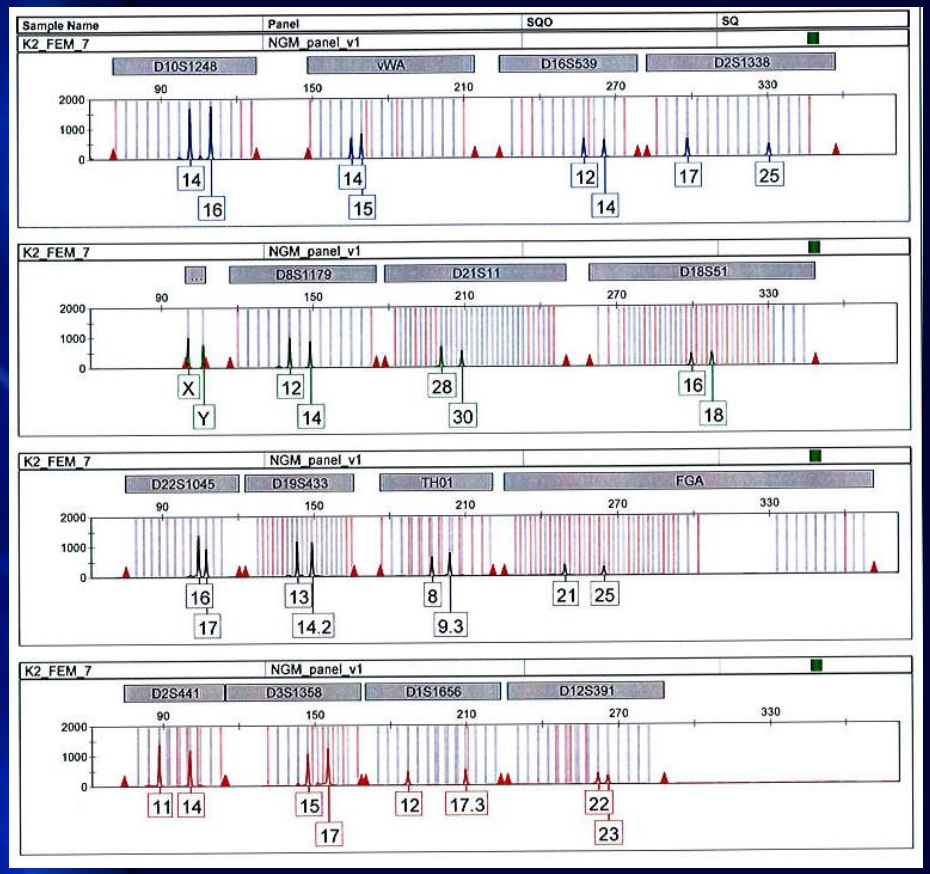
Autosomal genetic profiles of skeletal remains

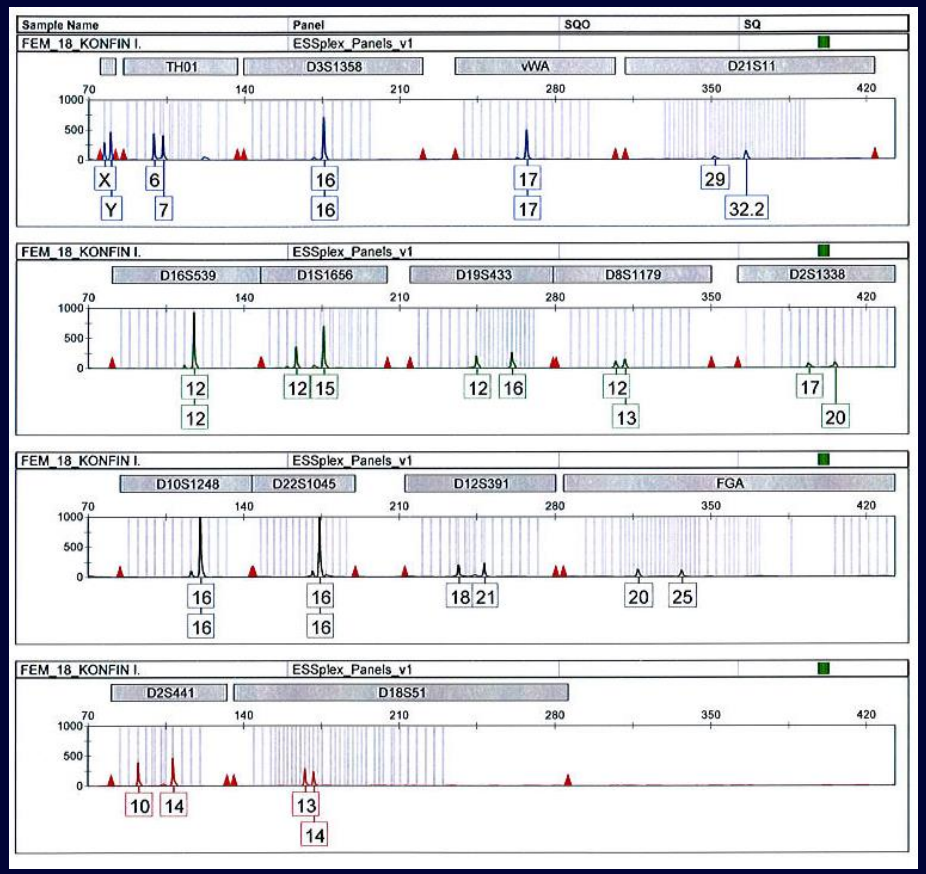






Autosomal genetic profiles of skeletal remains

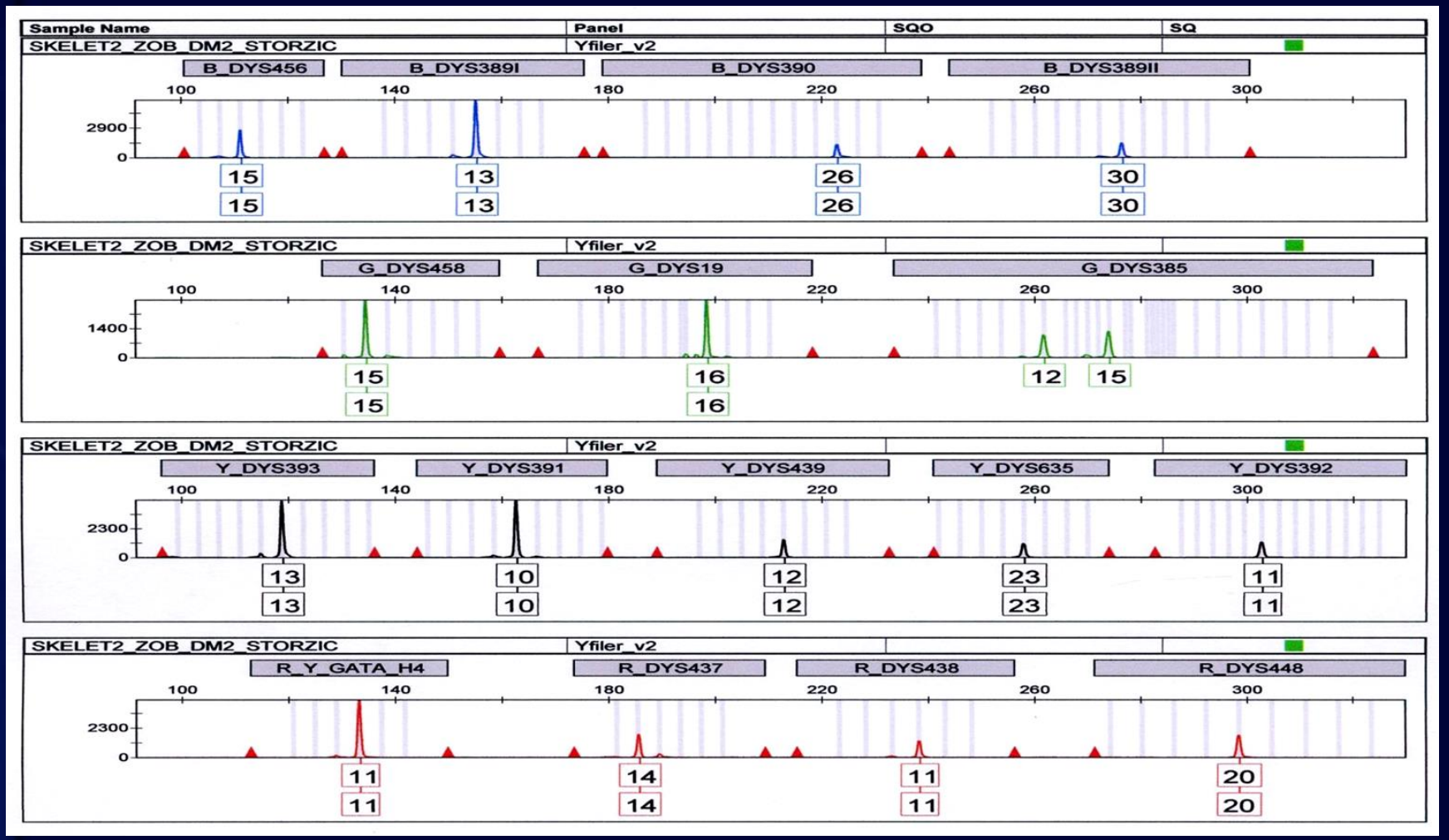




NGM

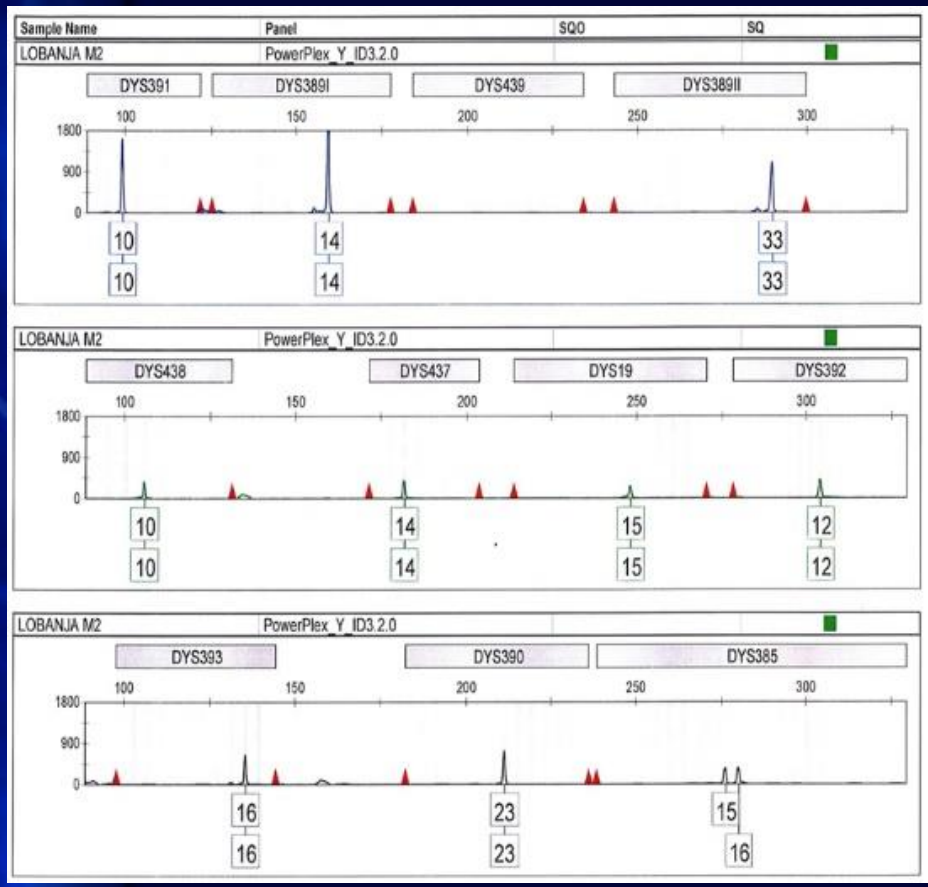
ESSplex Plus

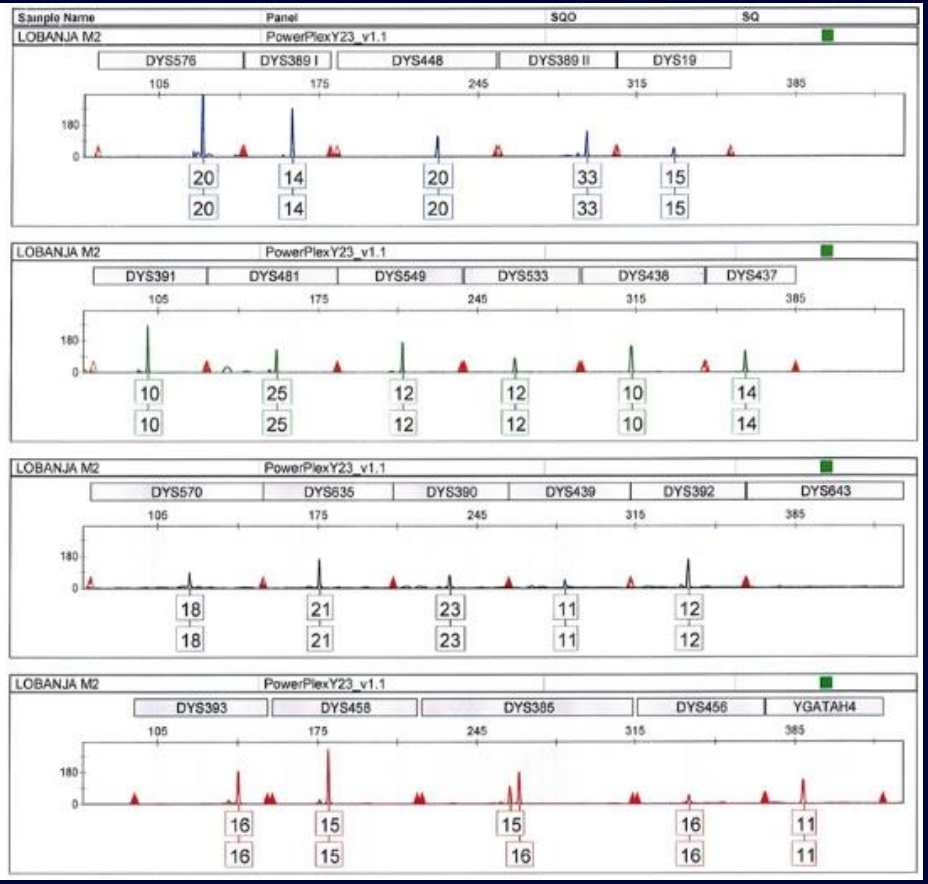
Y-STR genetic profile of skeletal remains



YFiler

Y-STR genetic profile of skeletal remains





PowerPlex Y

PowerPlex Y23

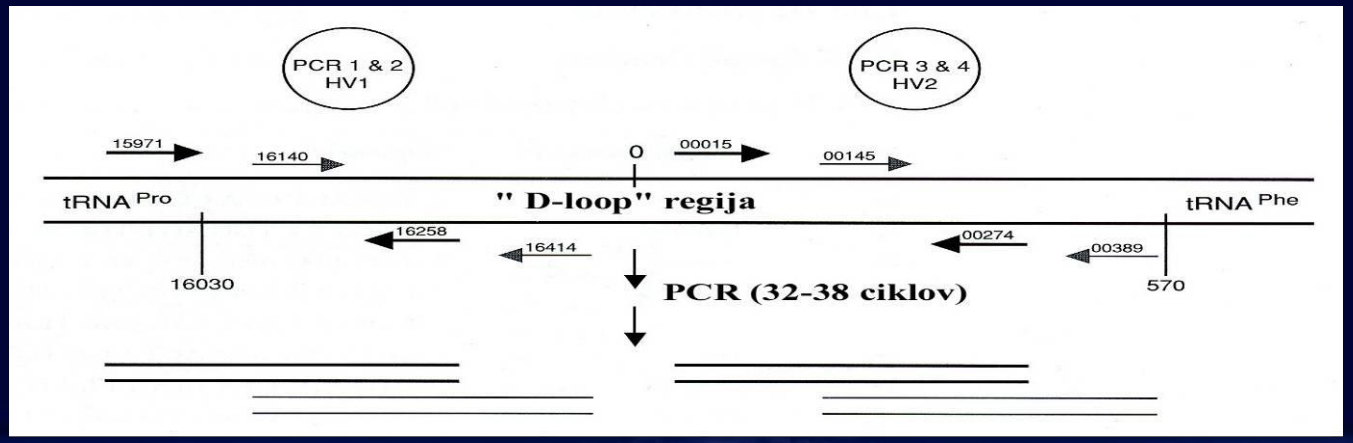
The current mtDNA analysis

Sequence 783 nucleotide bases on light and heavy strands of the mtDNA molecule: - HV1: 15996-16400 (~405 bases)

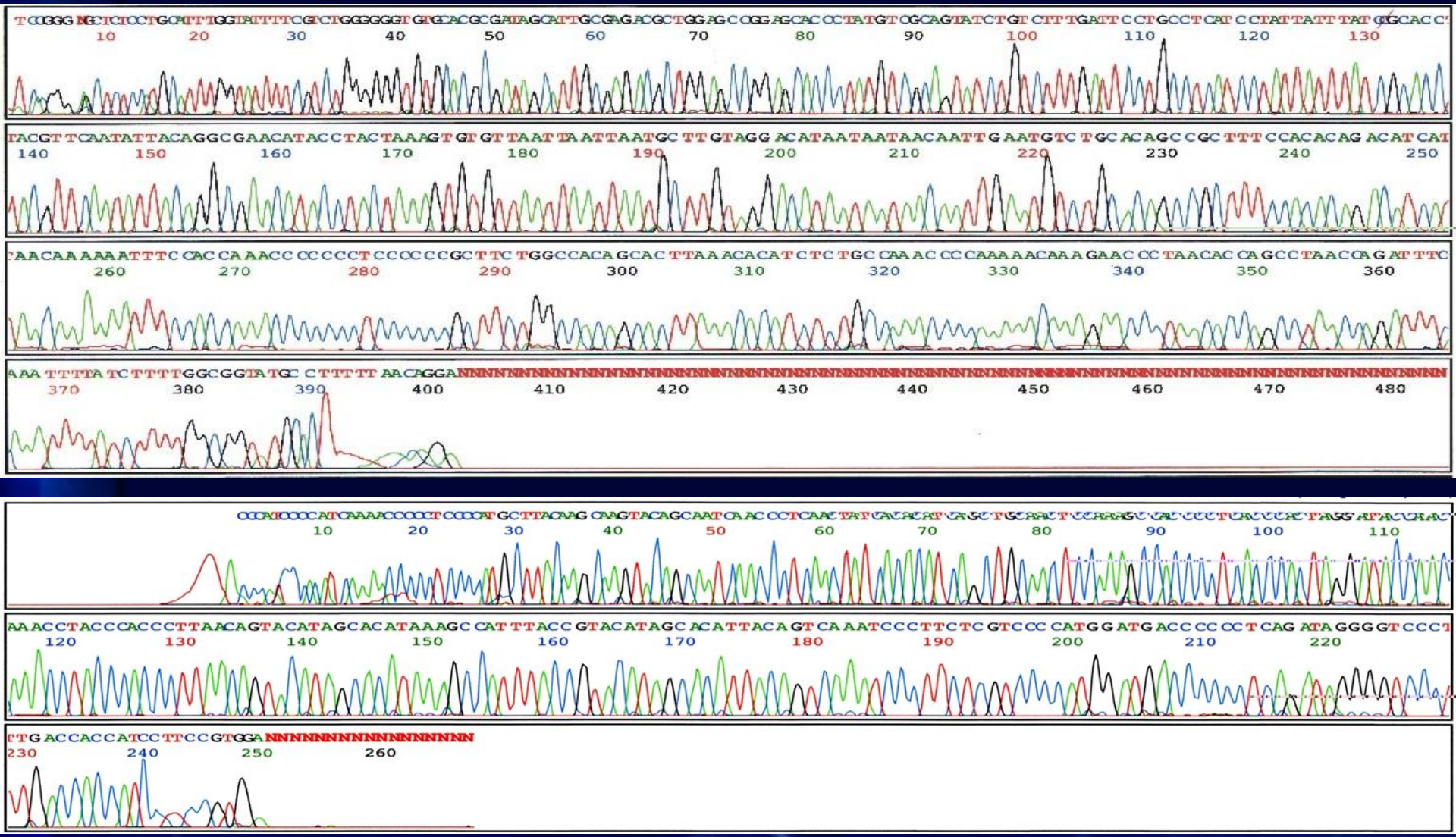
- HV2: 30-407 (~378 bases)

- Less degraded samples: PCR amplification of whole HVI and HVII

- More degraded samples: PCR amplification of two overlapping PCR fragments for HVI and HVII



Longer and shorter sequences



MtDNA analyses of WWII skeletal remains

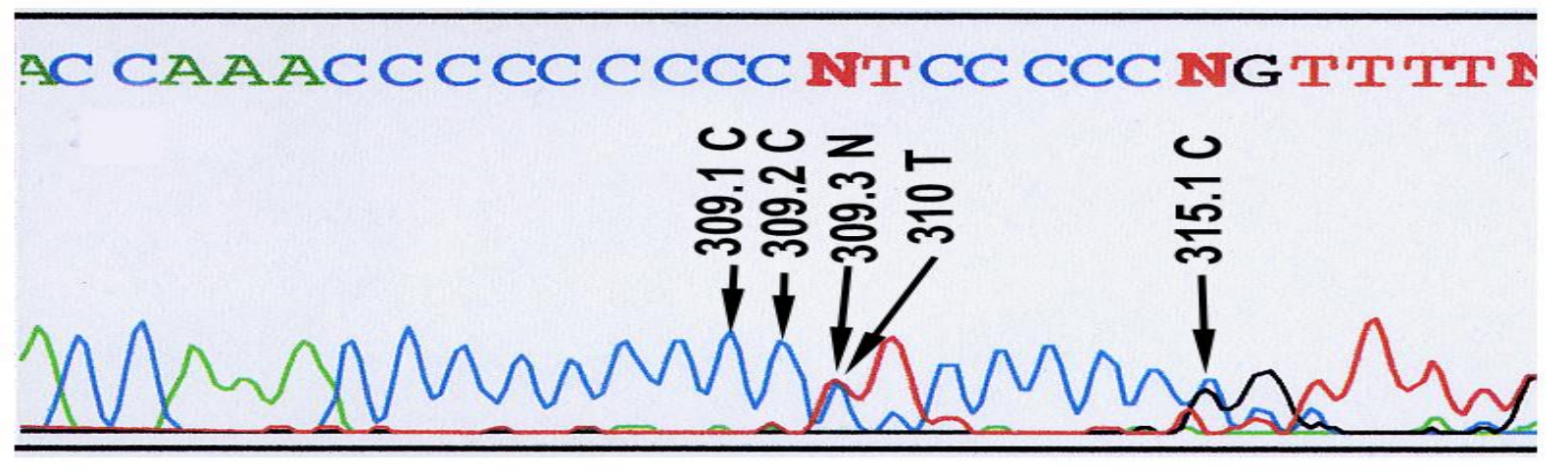
- increased amount of Taq polymerase in PCR
- addition of Bovine Serum Albumin in PCR (0.625 μg/μl)
- increasing the number of cycles from 32 to 38
- sequencing of light and heavy chain is necessary

MtDNA analyses of WWII skeletal remains

- duplication of analyses - two amplifications of two extractions (duplication is very important for determining of heteroplasmy)
- Heteroplasmy is presence of more than two types of mtDNA within an individual:
  - length heteroplasmy
  - point heteroplasmy

Length heteroplasmy

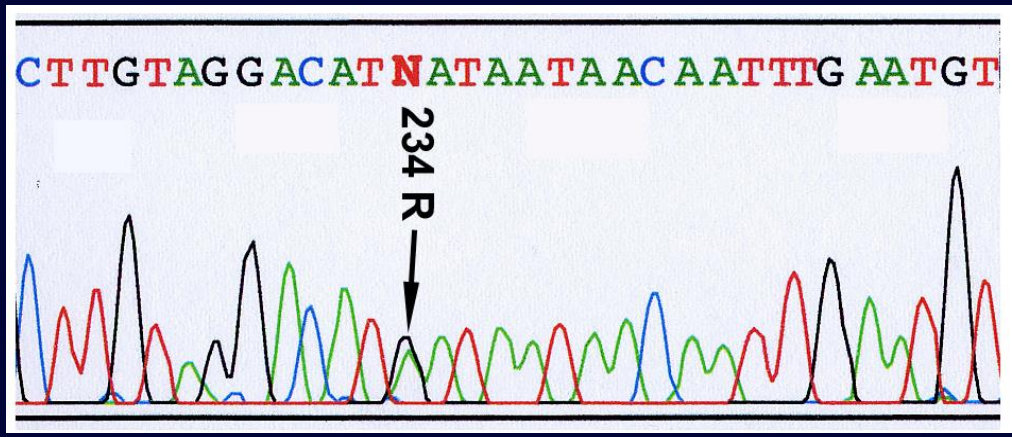
- HVI 17% in Slovenian population sample
- HVII 8% in Slovenian population sample

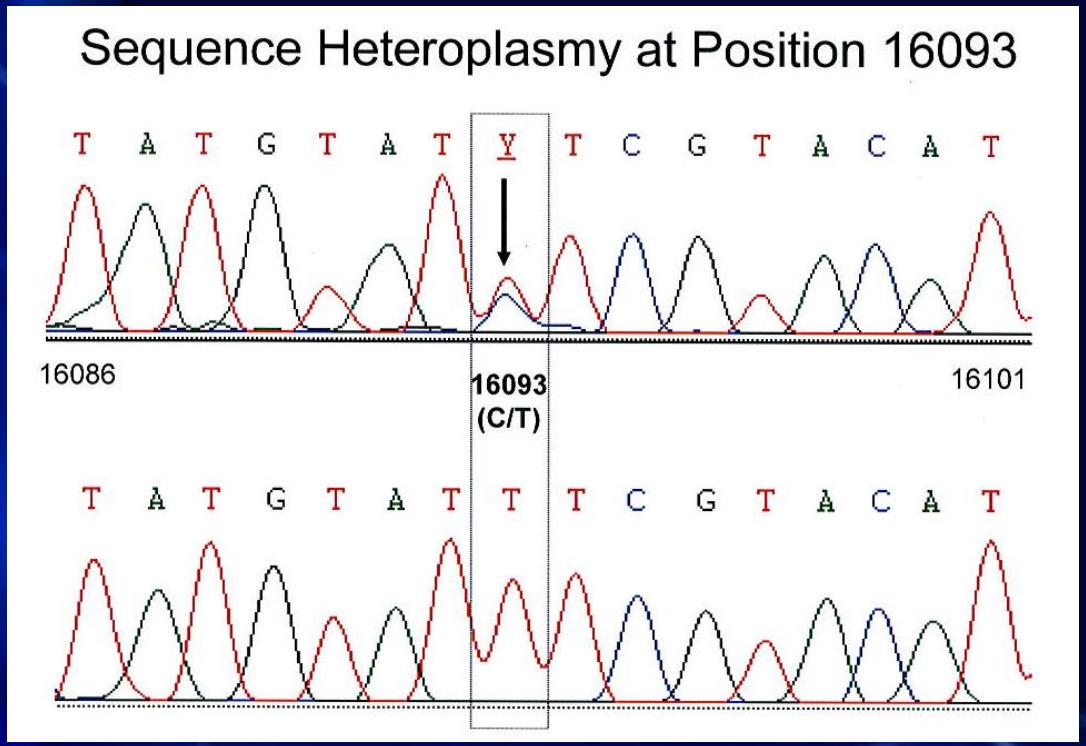


- length heteroplasmy in C-stretch region of HVII on position 303-315
- two mtDNA length variants:
    1. on position 309 insertion of two C nucleotides
    2. on position 309 insertion of three C nucleotides (dominant)

Point heteroplasmy

HVII: 234 (A+G) ; 234R

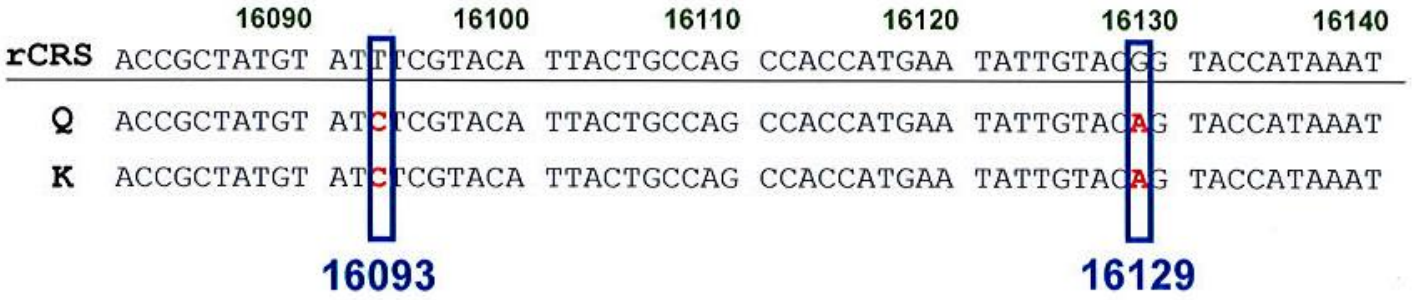




HVI: 16093 (C+T); 16093Y

Note differences from reference sequence (Anderson sequence or CRS)

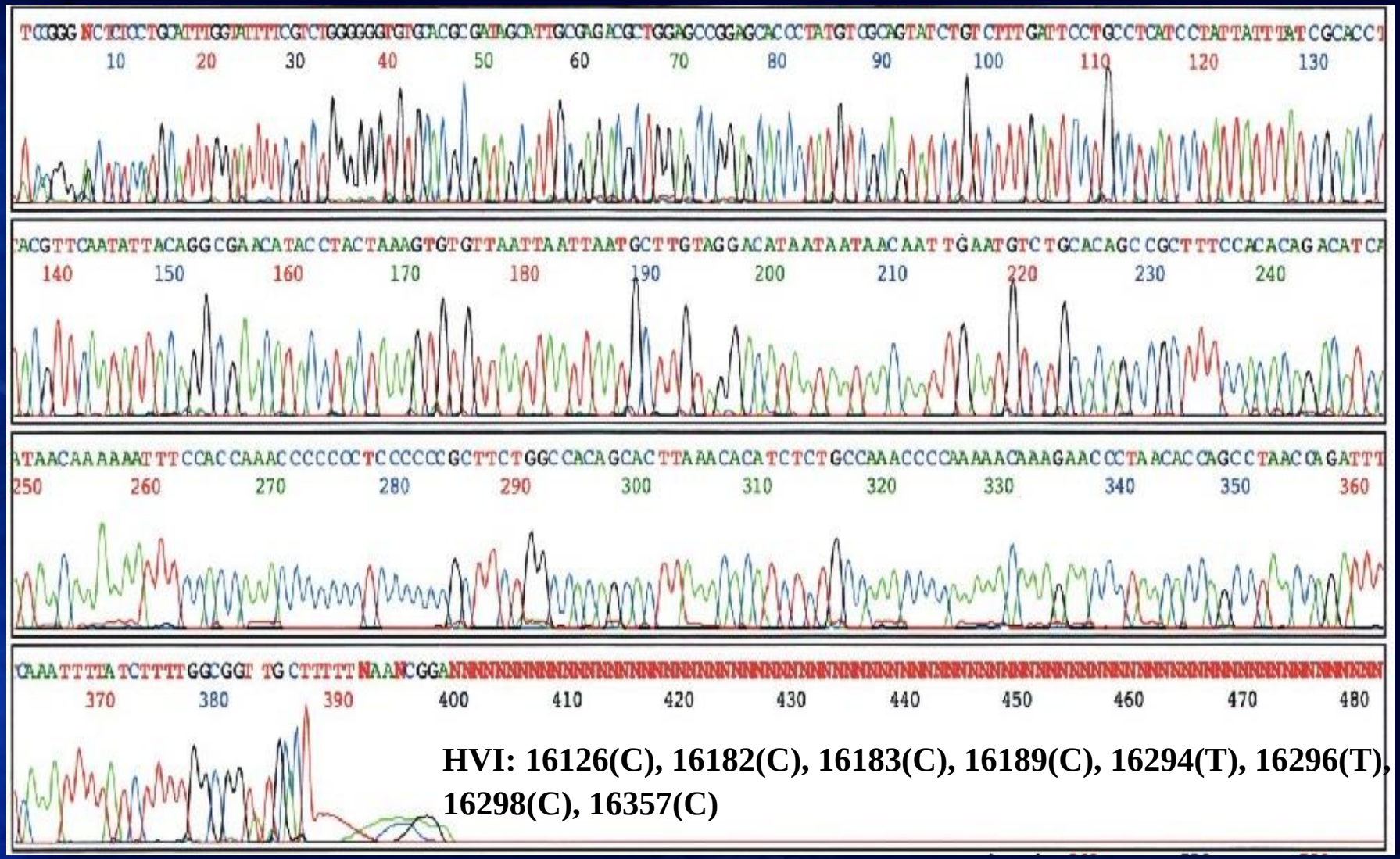
mtDNA sequences from tested samples are aligned with the reference rCRS sequence (e.g., positions 16071-16140)



Differences are reported by the position and the nucleotide change (compared to the rCRS)

Sample Q	Sample K
16093C	16093C
16129A	16129A

Determine the mtDNA sequence



HVI: 16126(C), 16182(C), 16183(C), 16189(C), 16294(T), 16296(T), 16298(C), 16357(C)

NGS technology

NGS ION S5

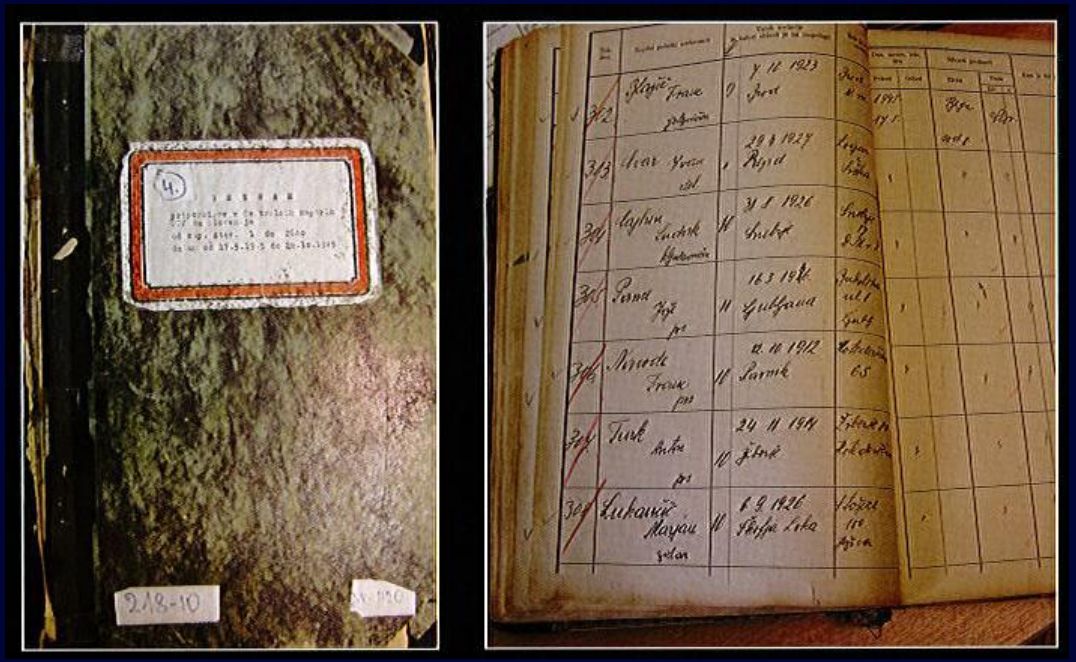
NGS ION CHEF

NGS Precision ID STR Panel

Locus	Genotype
AMELX	1
AMELY	
CSF1PO	11, 13
D10S1248	13, 14
D12ATA63	13, 17
D12S391	18, 23
D13S317	8, 9
D14S1434	13, 14
D16S539	11, 12
D18S51	11, 16
D19S433	14
D1S1656	11, 15
D1S1677	13, 18
D21S11	30, 32
D22S1045	16
D2S1338	21, 23
D2S1776	11, 12
D2S441	11, 15
D3S1358	14, 18
D3S4529	15
D4S2408	9, 11, 11
D5S2800	17
D5S818	10, 11
D6S1043	17, 18
D6S474	17
D7S820	10, 11
D8S1179	16, 16
DYS391	
FGA	19, 25
Penta_D	9, 10
Penta_E	13, 16
SRY	
TH01	7, 9
TPOX	11
Yindel	
vWA	14, 16

Genetic identification of Slovenian WWII mass grave victims

- It was possible only for mass graves where we could access lists of victims, based on which we were able to collect comparative samples of buccal swabs from living relatives:
  - Konfin I (88 skeletons)
  - Konfin II (62 skeletons)
  - Storžič (4 skeletons)
  - Bodovlje (25 skeletons)
  - Mozelj (5 skeletons)
  - Mačkovec (16 skeletons)
  - Zaplana (12 skeletons)
  - Kržeti (3 skeletons)
  - Mače (2 skeletons)
  - Babna Gora (7 skeletons)
  - Krimska jama (35 skeletons)

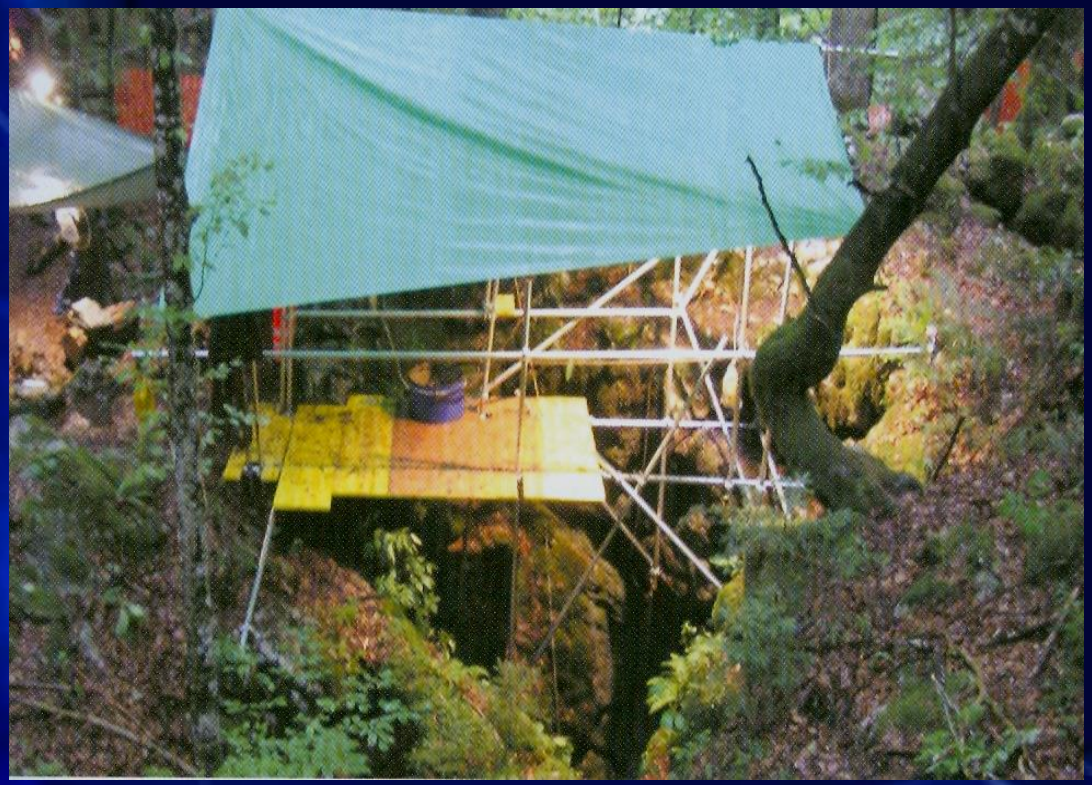


Identification of skeletal remains from mass grave at the cave site Konfin I

- 24th of June 1945 88 Slovenian men were executed without conviction of any crime in a court. Their bodies were thrown into 45-m-deep karst cave, and the entrance was dynamited

Konfin I mass grave

- The bodies were not covered with earth that would have kept the skeletons in their original position – excavation in anatomical position was not possible



Konfin I mass grave - excavation

- Excavation was performed in 2006 by arhaeologists
- Genetic identification was performed by our laboratory few years later
- Metal bucket for lifting the skeletal remains was used

Anthropological study

With the anthropological study the number, gender and age of the victims were determined. The anthropologist found out that all of the victims were male and that the number of the victims is between 85 and 89.

Selection of the bones for genetic analyses

- No teeth
- We selected all 84 right femurs for genetic typing (67 complete femurs and 17 proximal fragments)

Reference samples

We collected buccal swabs from 50 family references (sisters, brothers, daughters, sons, wives, cousins and nephews) that were close or distant relatives of 44 Konfin I mass grave victims:

- 7 brothers (autosomal and Y STRs and mtDNA)
- 20 sisters (autosomal STRs and mtDNA)
- 5 sons (autosomal and Y STRs)
- 8 daughters and wife (autosomal STRs)
- 2 maternal cousins (autosomaln STRs and mtDNA)
- 5 maternal nephews (autosomaln STRs and mtDNA)
- 3 paternal nephews (autosomaln and Y STRs)

Elimination database

- We created elimination database where all the profiles of 15 persons that came in contact with Konfin I. skeletons were stored. Elimination database included profiles of persons who excavated, stored and examined the skeletal remains in any stage of the process.

DNA typing of bones, relatives and persons to be included in elimination database

- DNA typing of bones (autosomal and Y-STRs and mtDNA for all bones)
- DNA typing of relatives (autosomal STRs for all of them, Y-STRs for paternal relatives and mtDNA for maternal relatives)
- DNA typing of persons to be included in elimination database (autosomal STRs and mtDNA for all of them and Y-STRs for males)

Results of genetic analyses of bones from Konfin I mass grave

- Quantification: we extracted 0.4 to 100 ng DNA/g of bone powder
- Autosomal genetic profiles: were obtained from 98% of the bones
- Y-chromosome haplotypes : were obtained from 98% of the bones
- mtDNA haplotypes: were obtained from 95% to 98% of the bones (HVI and HVII region)

Results of DNA typing of bones from Konfin I mass grave

Kost	Količina (pg/µl)	n-STR	Y-STR	mtDNA	Kost	Količina (pg/µl)	n-STR	Y-STR	mtDNA
femur A ant	221	18/18	17/17	HVI, HVII	D femur 30	64	18/18	17/17	HVI, HVII
femur B ant	32	18/18	7/17	HVI, HVII	D femur 31	17	16/18	13/17	HVI, HVII
femur C ant	60	17/18	17/17	HVI, HVII	D femur 32	42	18/18	17/17	HVI, HVII
femur D ant	37	18/18	17/17	HVI, HVII	D femur 33	100	18/18	17/17	HVI, HVII
femur E ant	70	18/18	17/17	HVI, HVII	D femur 34	62	18/18	16/17	HVI, HVII
femur F ant	50	18/18	14/17	HVI, HVII	D femur 35	64	18/18	10/17	HVI, HVII
femur G ant	40	17/18	12/17	HVI, HVII	D femur 36	77	16/18	16/17	HVI, HVII
femur H ant	106	18/18	17/17	HVI, HVII	D femur 37	280	18/18	17/17	HVI, HVII
femur I ant	26	17/18	17/17	HVI, HVII	D femur 38	130	18/18	17/17	HVI, HVII
D femur 43 p.	237	18/18	17/17	HVI, HVII	D femur 39	150	18/18	17/17	HVI, HVII
D femur 45 p.	46	17/18	14/17	HVI, HVII	D femur 40	30	17/18	17/17	
D femur 49 p.	116	18/18	17/17	HVI, HVII	D femur 41	50	18/18	17/17	HVI, HVII
D femur 51 p.	56	18/18	17/17	HVI, HVII	D femur 42	43	17/18	12/17	HVI, HVII
D femur 1	62	18/18	16/17	HVI, HVII	D femur 43	8	14/18	14/17	HVI, HVII
D femur 2	10	13/18	12/17		D femur 44	26	17/18	17/17	HVI, HVII
D femur 3	43	18/18	16/17	HVI, HVII	D femur 45	57	18/18	17/17	HVI, HVII
D femur 4	21	17/18	16/17	HVI, HVII	D femur 46	47	24/24	7/17	HVI, HVII
D femur 5	18	16/18	14/17	HVI, HVII	D femur 47	77	18/18	17/17	HVII
D femur 6	10	14/18	7/17	HVII	D femur 48	100	18/18	17/17	HVI, HVII
D femur 7	24	18/18	17/17	HVI, HVII	D femur 49	101	18/18	10/17	HVI, HVII
D femur 8	69	18/18	16/17	HVI, HVII	D femur 50	90	18/18	9/17	HVI, HVII
D femur 9	17	18/18	16/17	HVI, HVII	D femur 51	47	18/18	7/17	HVI, HVII
D femur 10	46	18/18	16/17	HVI, HVII	D femur 52	18	18/18	17/17	HVI, HVII
D femur 11	114	18/18	17/17	HVI, HVII	D femur 53	91	18/18	8/17	HVI, HVII
D femur 12	120	18/18	17/17	HVI, HVII	D femur 54	14	14/18	16/17	HVI, HVII
D femur 13	35	18/18	17/17	HVI, HVII	D femur 55	120	18/18	10/17	HVI, HVII
D femur 14	60	16/18	13/17	HVI, HVII	D femur 56	109	18/18	17/17	HVI, HVII
D femur 15	100	18/18	17/17	HVI, HVII	D femur 57	73	18/18	10/17	HVI, HVII
D femur 16	51	18/18	8/17	HVI, HVII	D femur 58	48	17/18	17/17	HVI, HVII
D femur 17	110	18/18	17/17	HVI, HVII	D femur 59	47	18/18	9/17	HVI, HVII
D femur 18	1000	18/18	17/17	HVI, HVII	D femur 60	24	18/18	16/17	HVI, HVII
D femur 19	270	18/18	17/17	HVI, HVII	D femur 61	250	18/18	17/17	HVI, HVII
D femur 20	15	11/18	14/17	HVI, HVII	D femur 62	33	18/18	14/17	HVI, HVII
D femur 21	142	18/18	17/17	HVI, HVII	D femur 63	17	17/18	16/17	HVI, HVII
D femur 22	111	18/18	17/17	HVI, HVII	D femur 64	18	18/18	15/17	HVI, HVII
D femur 23	130	18/18	17/17	HVI, HVII	D femur 65	4	0/18	0/17	HVI, HVII
D femur 24	75	18/18	17/17	HVI, HVII	D femur 66	36	18/18	17/17	HVI, HVII
D femur 25	36	18/18	17/17	HVI, HVII	D femur 67	34	18/18	17/17	HVI, HVII
D femur 26	39	16/18	15/17	HVI, HVII	D femur 68	5	0/18	0/17	HVI, HVII
D femur 27	150	18/18	16/17	HVI, HVII	D femur 69	93	18/18	7/17	HVI, HVII
D femur 28	41	18/18	17/17	HVI, HVII	D femur 70	15	22/24	11/17	HVI, HVII
D femur 29	48	15/18	6/17	HVI, HVII	D femur 71	51	17/18	16/17	HVI, HVII

Elimination database: DNA typing of autosomal STR

Bones genetic profiles were compared with autosomal, Y-STR and mtDNA genetic profiles of elimination database persons

Vzorec	D8S1179		D21S11		D7S820		CSF1PO		D3S1358		THO1		D13S317		D16S539	
P. L. S.	14	14	30.2	30.2	10	12	11	12	15	17	8	9	12	13	8	12
I. Z. P.	13	15	30	32.2	9	10	9	11	14	18	6	9	11	11	11	12
B.G.P.	10	14	29	31	10	11	12	12	14	17	7	9.3	8	11	9	9
K. V.	13	16	28	30	8	10	10	11	16	16	8	9	11	13	9	12
K. I.	12	13	30	31.2	8	8	12	13	18	18	8	9.3	9	11	12	12
G. M.	12	14	28	29	9	12	11	12	14	15	6	6	11	12	12	12
R. B.	13	14	30	32.2	10	11	9	12	15	16	6	9.3	10	12	10	12
An. M.	10	12	28	30	8	10	9	11	15	17	9	9	12	13	12	12
D.J.	13	15	28	29	8	10	10	11	14	15	9.3	9.3	11	12	11	13
P.P.	13	14	28	29	9	9	11	11	14	15	6	9.3	12	12	12	12
A.S.S.	10	13	29	30	10	11	11	12	15	17	6	9	11	12	10	11
P.R.	15	15	30	30	8	13	11	13	16	17	8	9.3	11	11	11	12
D.H.	10	11	29	29	8	11	10	11	17	19	6	9.3	12	12	8	12
Al.M.	11	15	30	32.2	10	12	11	12	15	15	6	9	8	11	11	12
P.J.	12	12	28	28	11	11	9	12	16	18	6	9.3	11	12	9	12
Vzorec	D2S1338		D19S433		vWA		TPOX		D18S51		Amelog.		D5S818		FGA	
P. L. S.	17	25	14	14	16	16	11	11	15	18	X	X	12	13	21	24
I. Z. P.	24	26	15	16	17	18	8	11	12	16	X	X	12	12	22	24
B. G. P.	17	20	13	15.2	14	16	8	12	10	17	X	X	11	11	20	20
K. V.	17	25	13	14	18	18	8	11	17	19	X	X	11	13	19	20
K. I.	20	24	13	13	14	17	8	10	14	17	X	X	13	14	22	24
G. M.	17	17	14	16	17	17	8	9	12	18	X	Y	12	13	20	21
R. B.	17	21	14	14	14	19	8	9	14	19	X	Y	11	12	21	24
An. M.	17	25	13	14	16	17	8	11	12	15	X	Y	12	12	24	25
D.J.	17	19	14.2	16	14	16	8	9	16	19	X	Y	11	12	21	23
P.P.	18	24	14	14	16	17	8	9	12	15	X	Y	12	13	18	25
A.S.S.	18	20	14	14	17	17	11	11	17	21	X	Y	10	12	19	22
P.R.	18	20	13	13.2	17	18	8	8	12	17	X	Y	12	12	19	25
D.H.	17	20	14	16	18	19	11	11	13	16	X	Y	11	12	22	24
Al.M.	17	19	15	15.2	18	18	11	12	15	18	X	Y	11	11	21	23.2
P.J.	20	23	13	15	17	18	8	8	13	18	X	Y	11	12	21	23

Vzorec	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385a/b	DYS393
G. M.	15	14	24	31	17	16	14/15	13
R. B.	15	13	24	30	16	17	14/15	13
An. M.	17	13	25	30	15	16	11/14	13
D.J.	17	13	25	30	16	13	16/18	13
P.P.	15	12	22	28	15	14	13/15	13
A.S.S.	15	13	25	29	17	16	14/15	13
P.R.	17	14	25	31	14	16	11/14	13
D.H.	16	13	25	31	15	15	11/14	13
Al.M.	17	13	25	30	16	16	11/14	13
P.J.	15	13	24	31	18	15	14/15	13
Vzorec	DYS391	DYS439	DYS635	DYS392	DYSH4	DYS437	DYS438	DYS448
G. M.	11	13	23	11	11	15	10	20
R. B.	11	13	23	11	11	15	10	20
An. M.	10	10	23	11	12	14	11	20
D.J.	10	11	23	11	12	14	10	20
P.P.	10	11	21	11	10	16	10	20
A.S.S.	11	13	23	11	11	15	10	20
P.R.	11	10	23	11	12	14	11	20
D.H.	10	12	23	11	11	14	11	20
Al.M.	10	10	23	11	12	14	11	20
P.J.	11	13	23	11	11	15	10	20

Vzorec	Razlike glede na "CRS"	Območje
P. L. S.	HVI: 16298C HVII: 72C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407
I. Z. P.	HVI: 16343G HVII: 73G, 150T, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
B. G. P.	HVI: 16126C, 16182C, 16183C, 16189C, 16294T, 16296T, 16298C, 16357C HVII: 73G, 195C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
K. V.	HVI: 16298C HVII: 72C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
K. I.	HVI: 16311C, 16362C HVII: 239C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
G. M.	HVI: 16362C HVII: 239C, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
R. B.	HVI: identična CRS HVII: 152C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407
An. M.	HVI: 16069T, 16126C HVII: 73G, 185A, 188G, 228A, 263G, 295T, 315.1C	HVI: 16030-16400 HVII: 55-407
D.J.	HVI: 16261T HVII: 200G, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
P.P.	HVI: 16126C, 16294T, 16296T, 16304C HVII: 73G, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
A.S.S.	HVI: 16298C HVII: 72C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
P.R.	HVI: 16362C, 16400T HVII: 239C, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
D.H.	HVI: 16126C, 16292T, 16294T, 16296T, 16304C HVII: 73G, 263G, 309.1C, 315.1C, 321C	HVI: 16030-16400 HVII: 55-407
Al.M.	HVI: 16126C, 16294T, 16296T, 16304C HVII: 73G, 263G, 309.1C, 309.2C, 315.1C	HVI: 16030-16400 HVII: 55-407
P.J.	HVI: 16170G, 16390A HVII: 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407

Excluding the possibility of contamination for Konfin I mass grave skeletal remains

- The cleanliness of the extraction blind control and amplifying negative control were checked and they were clean
- At least two separate analyses (from extraction to DNA typing) of the same bone gave the same results
- In the elimination database there were no identical a-STR, Y-STR and mtDNA genetic profile

Comparison of bone genetic profiles with family references

- comparison of mtDNA haplotypes (full match with maternal relatives is needed)
- comparison of Y-STR haplotypes (full match with paternal relatives is needed)
- comparison of autosomal STR profiles - recombination (daughters and sons at least 50 % common alleles with their fathers – victims – DNA VIEW)

Interpretation of results - LR and PP calculation

- Likelihood ratio (LR):

- LR tells us how many more likely it is that victims' bones are related to the family references, rather than to unrelated individual
- For autosomal STR profiles LR for kinship analyses is calculated using DNA VIEW - C. Brenner
- for Y-STR and mtDNA haplotypes for LR calculation haplotype frequency is estimated using Y-STR and mtDNA databases. The counting method is used to estimate the haplotype frequencies and a 95 % confidence interval

  - Y chromosome haplotype reference database - YHRD

  - European mtDNA database - EMPOP

LR = 1 / haplotype frequency

Interpretation of results - LR and PP calculation

- Posterior probability (PP):

  PP = LR x prior / (LR x prior + (1 – prior)) x 100%

  Prior probability (prior) = 1/n+1

(n = number of victims in mass grave) Prior = 1/89 = 0.01

Following recommendations (Biesecker et al. 2005; Brenner and Weir 2003; Prinz et al. 2007), the prior probability is set based on the number of mass grave victims reported, and a recommended posterior probability for kinship of 99.9% is used with the goal of high confidence of correct identification of victims in the mass grave

kinship analyses:
BROTHER/
SISTER
(DNA VIEW
C. Brenner)

For positive identification, the posterior probability has to be 99.9%
We combined autosomal STRs with mtDNA haplotypes to reach that value

Institute of Forensic Medicine 2009/7/22 13:15
DNAVIEW ver 28.48 2007/9/2 11:52

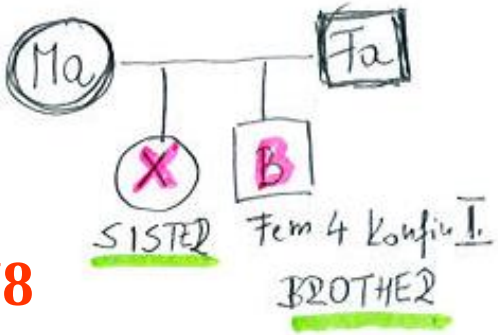
Case 930099
X,B:Ma+Fa
/Other,B:Ma+Fa
C

BROTHER/SISTER

D8S1179	0,25	1 / 4		
D21S11	1,36	(1+p) / 4p		p=0.226
D7S820	1,17	(1+r) / 4r		r=0.271
CSF1PO	15,3	(1+p+s+2ps) / 8ps		p=0.0337 s=0.339
D3S1358	0,771	(1+2q) / 8q		q=0.24
THO1	1,45	(1+p) / 4p		p=0.209
D13S317	11,7	(1+p+s+2ps) / 8ps		p=0.349 s=0.0434
D16S539	0,702	(1+2p) / 8p		p=0.277
D2S1338	0,25	1 / 4		
D19S433	0,61	(1+2r) / 8r		r=0.347
VWA31	3,22	(1+p+q+2pq) / 8pq		p=0.282 q=0.225
TPOX	2,03	(1+p+s+2ps) / 8ps		p=0.569 s=0.221
D18S51	1,69	(1+p) / 4p		p=0.173
D5S818	5,87	(1+p+r+2pr) / 8pr		p=0.0898 r=0.359
FGA	1,84	(1+p) / 4p		p=0.158
Penta D	1,3	(1+2p) / 8p		p=0.119
Penta E	5,04	(1+u) / 4u		u=0.0522

cumulative LR 6660
Posterior probability=98,7% assuming prior=1/89 $= \frac{n}{n+1} = \frac{1}{88+1} = \frac{1}{89}$

PRIOR = 0,01



LR mtDNA=4778

kinship analyses: NEPHEW/ UNCLE – paternal line (DNA VIEW C. Brenner)

For positive identification, the posterior probability has to be 99.9% We combined autosomal STRs with Y-STR haplotypes to reach that value

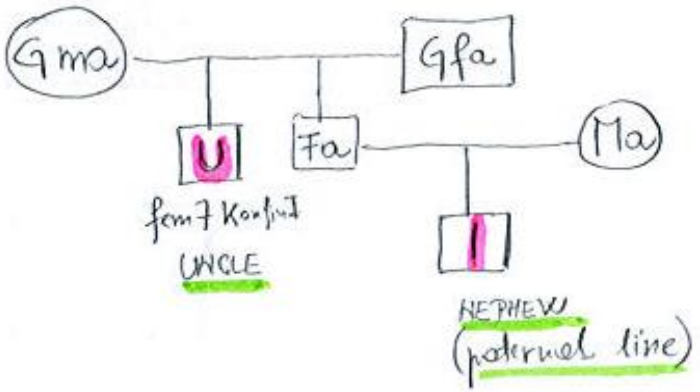
Institute of Forensic Medicine -- 2011/5/9 11:58
DNAVIEW ver 29.48 2011/3/8 10:38

NEPHEW / UNCLE

```
Case 730099
I:Ma+Fa
U,Fa:Gma+Gfa
/I:Ma+Fa
/Other,Fa:Gma+Gfa
c
D21S11          0,5       1 / 2
D7S820          1,42      (1+2p) / 4p          p=0.271
CSF1PO          0,5       1 / 2
D3S1358         1,02      (1+4q) / 8q          q=0.24
THO1            2,64      (1+2p) / 4p          p=0.117
D13S317         1,22      (1+2p) / 4p          p=0.349
D16S539         0,908     (1+4q) / 8q          q=0.306
D2S1338         9,43      (p+u+4pu) / 8pu      p=0.056 u=0.0187
D19S433         0,5       1 / 2
VWA31           2,36      (1+4s) / 8s          s=0.0674
TPOX            0,939     (1+2p) / 4p          p=0.569
D18S51          0,5       1 / 2
D5S818          1,21      (p+q+4pq) / 8pq      p=0.348 q=0.359
FGA             1,29      (1+4p) / 8p          p=0.158
D8S1179         0,862     (1+4r) / 8r          r=0.345
cumulative LR 7,42
Posterior probability=7% assuming prior=1/100
```

1/89 · 0,01

PRIOR = 0,01



kinship analyses: COUSINS- paternal line (DNA VIEW– C. Brenner)

For positive identification, the posterior probability has to be 99.9% We combined autosomal STRs with Y-STR haplotypes to reach that value

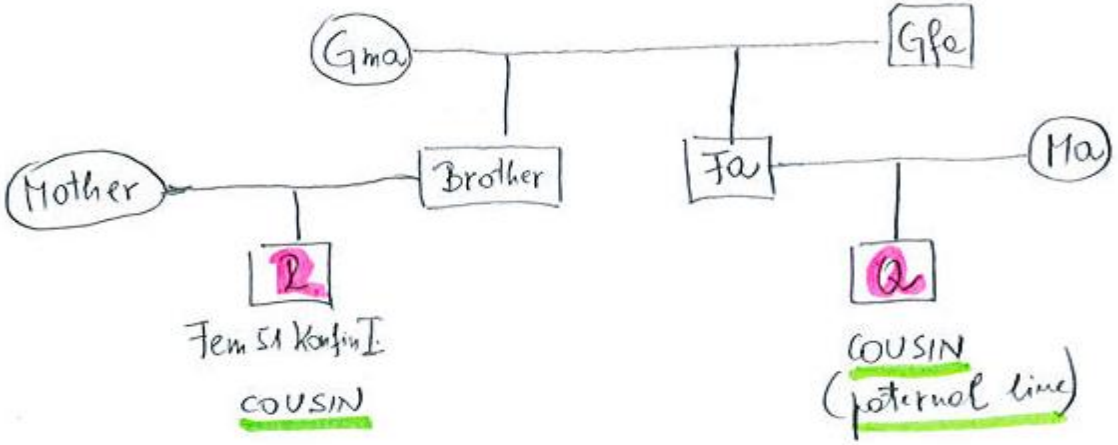
Institute of Forensic Medicine -- 2011/2/23 12:12
DNAVIEW ver 29.46 2011/1/16 7:34

```
Case 1130099
Q:Fa+Ma
R:brother+mother
brother,Fa:Gma+Gfa
/Q:Fa+Ma
/R:brother+mother
/Other,Fa:Gma+Gfa
c
D21S11          0,75      3 / 4
D7S820          1,16      (1+12p) / 16p          p=0.154
CSF1PO          2,61      (1+12p) / 16p          p=0.0337
D3S1358         1,3       (1+6q) / 8q            q=0.227
THO1            0,977     (1+12a) / 16a          a=0.276
D13S317         1,47      (1+3p) / 4p            p=0.349
D16S539         1,37      (1+6r) / 8r            r=0.201
D2S1338         1,37      (1+12v) / 16v          v=0.101
D19S433         1,11      (1+6p) / 8p            p=0.347
VWA31           0,75      3 / 4
TPOX            0,75      3 / 4
D18S51          1,19      (1+12p) / 16p          p=0.141
D5S818          1,1       (p+q+12pq) / 16pq      p=0.348 q=0.359
FGA             1,1       (1+12r) / 16r          r=0.179
D8S1179         0,931     (1+12q) / 16q          q=0.345
cumulative LR 6,66
Posterior probability=6,3% assuming prior=1/100
```

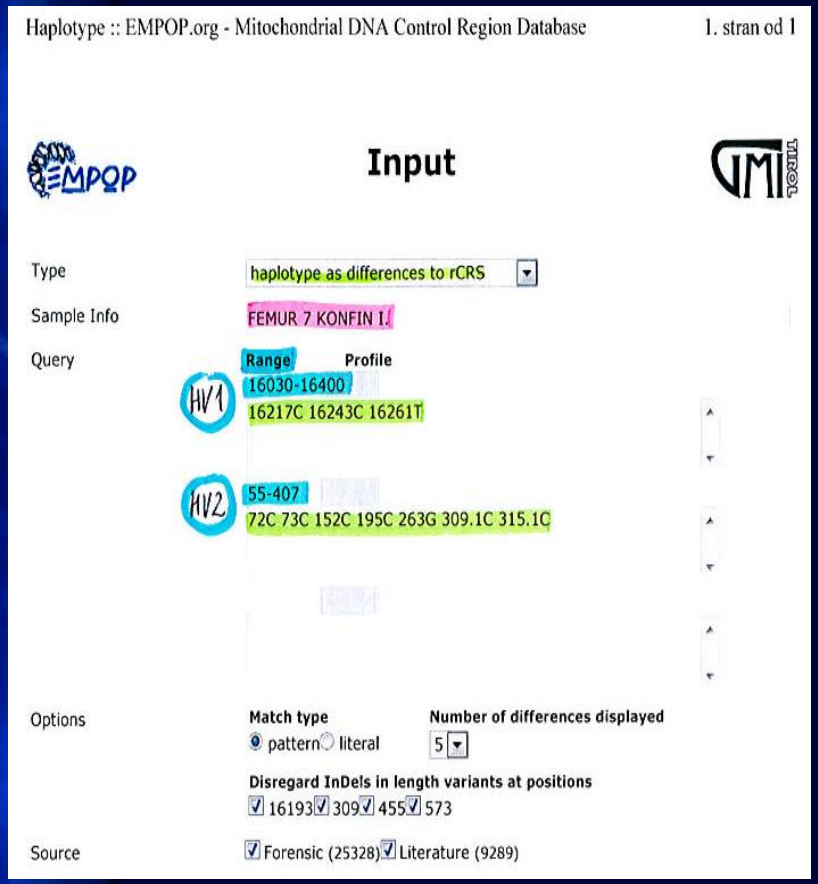
COUSINS

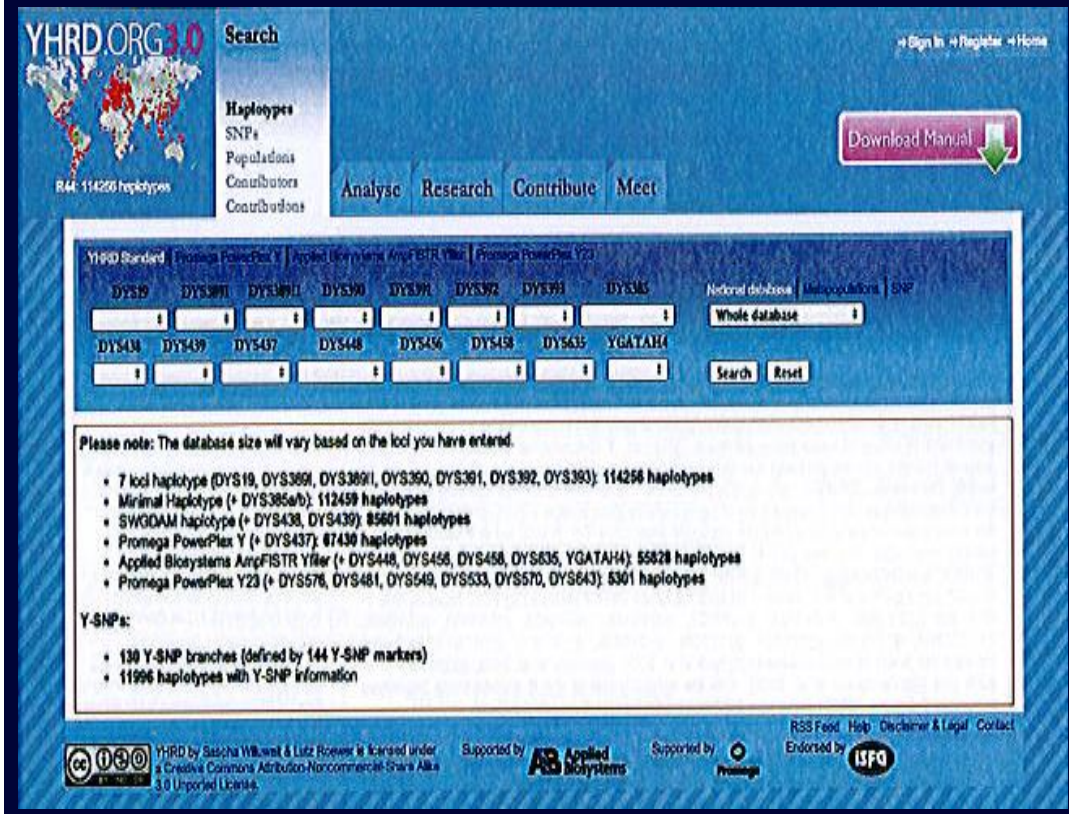
1/89 = 0,01

PRIOR = 0,01



A database context is needed for Y-STR and mtDNA haplotype frequency estimation





EMPOP database

EMPOP

Summary

Results Identification	382d3c84f7aa9ea4d31828fd118f5062c867abdc
Search execution date	2014-05-23 09:46:14 UTC
Sample Info	FEMUR 7 KONFIN I.
Type	string-based search: haplotype as differences to rCRS
Options	Match type: **pattern** Maximum differences displayed: **5** Disregard InDels in length variants at positions: **16193 309 455 573**
Source	**Forensic data (23503/25328)** **Literature data (4313/9289)**
Query	HV1 16030-16400 T16217CT16243CC16261T HV2 55-407 T72CA73CT152CT195CA263G-309.1C-315.1C
Filter	Geographic affiliation: Europe Metapopulation: **Westeurasian->European**

DIFFERENCES TO QUERY PROFILE	NUMBER OF HAPLOTYPES	CUMULATIVE NUMBER OF HAPLOTYPES
0	0	0
1	0	0
2	0	0
3	0	0
4	0	0
5	6	6
6+	4771	4777

Frequency estimates

P_{Uc}	**0.000e+0** [0.000e+0 ; 8.035e-4]
P_{N+1}	**2.093e-4** [3.694e-5 ; 1.185e-3]

EMPOP database - frequency estimation

Frequency estimates

P_{Uc}	**0.000e+0**	[0.000e+0 ; 8.035e-4]
P_{N+1}	**2.093e-4**	[3.694e-5 ; 1.185e-3]

LR = 1 / haplotype frequency

LR = 1/0.0002093 = 4778

The frequency estimates following two different approaches together with their confidence intervals are calculated as follows:
P_{Uc} denotes the uncorrected frequency k/n where k is the number of hits and n is the samplesize.
P_{N+1} denotes the N+1 counting method following the formula (k+1)/(n+1) for estimating the frequency.
For each of the estimated frequencies the confidence interval is computed following the approach of Wilson 1927:
With

$$c = \Phi^{-1}(1 - \frac{\alpha}{2})$$

where Phi denotes the normal distribution and alpha is set to 0.05 we get c=1.96. Let $\hat{p}$ be the estimated frequency, then

$$p_{o,u} = \frac{1}{1 + \frac{c^2}{n}} \cdot \left(\hat{p} + \frac{c^2}{2n} \pm c \cdot \sqrt{\frac{\hat{p} \cdot (1 - \hat{p})}{n} + \frac{c^2}{4n^2}} \right)$$

denotes the Wilson interval.

YHRD database - frequency estimation

Result

19	3891	3891	390	391	392	393	385	438	439	437	448	456	458	635	YGATAH4	576	481	549	533	570	643	Database
16	13	30	26	10	11	13	12,15	11	12	14	20	15	15	23	11							Whole database

- All Metapopulation: Found 0 of 71234 matching haplotypes [f=0 (95% CI: 0 – 5.178×10^{-5})] in 0 of 477 populations.
  - Eurasian Metapopulation: Found 0 of 33343 matching haplotypes [f=0 (95% CI: 0 – 1.106×10^{-4})] in 0 of 225 populations.
    - European Metapopulation: Found 0 of 25628 matching haplotypes [f=0 (95% CI: 0 – 1.439×10^{-4})] in 0 of 148 populations.
      - Western European Metapopulation: Found 0 of 15544 matching haplotypes [f=0 (95% CI: 0 – 2.373×10^{-4})] in 0 of 87 populations.
      - Eastern European Metapopulation: Found 0 of 4880 matching haplotypes [f=0 (95% CI: 0 – 7.556×10^{-4})] in 0 of 31 populations.
      - South-Eastern European Metapopulation: Found 0 of 2754 matching haplotypes [f=0 (95% CI: 0 – 1.339×10^{-3})] in 0 of 22 populations.

[f=0 (95% CI: 0 – 1.439×10^{-4})]

LR = 1 / haplotype frequency

LR = 1/0.0001439 = 6949

Combining different genetic markers (LR combined calculation)

The product rule is used to estimate a combined likelihood ratio:

- autosomal genetic profiles and mtDNA haplotypes:

  LRc = LR (autosomal STRs) × LR (mtDNA)

  (Castella et al. 2006)

- autosomal genetic profiles and Y-STR haplotypes:

  LRc = LR (autosomal STRs) × LR (Y-STRs)

  (Walsh et al. 2008)

Combining different genetic markers (PP combined calculation)

Posterior probability combined (PPc):

PPc = LRc x prior / (LRc x prior + (1 – prior)) x 100%

Prior probability (prior) = 1/n+1

(n = number of victims in mass grave)

Prior = 1/89 = 0,01 (Konfin I. mass grave)

The victim is identified with a high confidence of correct identification if posterior probability is higher than 99.9%

Identification of victim with living sister (combining of autosomal and mtDNA results)

- $LR_{(mtDNA)}$ = 4778 (PP=97.9%)

Vzorec	Razlike glede na "CRS"	Območje
FEMUR D ant	HVI: 16304C, 16311C HVII: 207A, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407
G. KERVINA	HVI: 16304C, 16311C HVII: 207A, 263G, 315.1C	HVI: 16030-16400 HVII: 55-407

- $LR_{(a\text{-}STR)}$ = 6660 (PP=98.7%)

- $LR_{(a\text{-}STR \times mtDNA)} = 3.2 \times 10^7$ (PP_c = 99,9997 %)

Vzorec	D8S1179		D21S11		D7S820		CSF1PO	
FEMUR D ant	10	13	29	31	9	10	12	12
G. KERVINA	10	13	29	31	9	12	12	12
Vzorec	**D3S1358**		**THO1**		**D13S317**		**D16S539**	
FEMUR D ant	16	17	8	10	10	13	12	12
G. KERVINA	16	17	8	10	11	11	12	13
Vzorec	**D2S1338**		**D19S433**		**vWA**		**TPOX**	
FEMUR D ant	23	24	13	13	14	16	8	11
G. KERVINA	23	24	13	13	14	17	8	11
Vzorec	**D18S51**		**Amelog.**		**D5S818**		**FGA**	
FEMUR D ant	13	15	X	Y	9	13	16	23.2
G. KERVINA	15	16	X	X	11	13	16	23.2

Identification of victim with living brother (combining of autosomal and mtDNA results and autosomal and Y-STR results)

- $LR_{(mtDNA)} = 2x10^3$ (PP=95.3%)

Vzorec	Razlike glede na "CRS"	Območje
D FEMUR 49 p.	HVI: 16192T, 16259T, 16270T HVII: 73G, 150T, 195C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407
V. ZORKO	HVI: 16192T, 16259T, 16270T HVII: 73G, 150T, 195C, 263G, 309.1C, 315.1C	HVI: 16030-16400 HVII: 55-407

Vzorec	D8S1179		D21S11		D7S820		CSF1PO	
D FEMUR 49 p.	10	14	28	30	8	9	12	14
V. ZORKO	10	14	28	30	8	8	12	12
Vzorec	**D2S1338**		**D19S433**		**vWA**		**TPOX**	
D FEMUR 49 p.	17	20	14	14	15	17	8	8
V. ZORKO	17	21	14	14	15	17	8	8
Vzorec	**D3S1358**		**THO1**		**D13S317**		**D16S539**	
D FEMUR 49 p.	15	16	6	9.3	11	14	12	12
V. ZORKO	15	15	6	6	11	14	11	12
Vzorec	**D18S51**		**Amelog.**		**D5S818**		**FGA**	
D FEMUR 49 p.	12	14	X	Y	11	12	19	23
V. ZORKO	13	16	X	Y	12	12	19	21

- $LR_{(a\text{-}STR)} = 2x10^4$ (PP=99.5%)
- $LR_{(Y\text{-}STR)} = 3x10^3$ (PP=96.8%)
- $LR_{(a\text{-}STR \times mtDNA)} = 5x10^7$ (PPc = 99,9998 %)
- $LR_{(a\text{-}STR \times Y\text{-}STR)} = 6x10^7$ (PPc = 99,9998 %)

Vzorec	DYS456	DYS389I	DYS390	DYS389II
D FEMUR 49 p.	15	13	24	32
V. ZORKO	15	13	24	32
Vzorec	**DYS458**	**DYS19**	**DYS385a/b**	**DYS393**
D FEMUR 49 p.	17	15	14/14	13
V. ZORKO	17	15	14/14	13
Vzorec	**DYS391**	**DYS439**	**DYS635**	**DYS392**
D FEMUR 49 p.	11	13	23	11
V. ZORKO	11	13	23	11
Vzorec	**DYSH4**	**DYS437**	**DYS438**	**DYS448**
D FEMUR 49 p.	11	15	10	20
V. ZORKO	11	15	10	20

Identified victims of Konfin I. mass grave

- 6 victims were identified with brothers (a-STR, Y-STR and mtDNA)
- 2 victims were identified with sons (a-STR and Y-STR)
- 15 victims were identified with sisters (a-STR and mtDNA)
- 1 victim was identified with maternal cousin (a-STR and mtDNA)
- 3 victims were identified with maternal nephews (a-STR and mtDNA)
- 4 victims were identified with douthers (a-STR)
- 1 victim was identified with douther and wife (a-STR)

Identified victims of Konfin I mass grave

- With combining close and dinstinc relatives and analysing nuclear, Y-chromosome and mtDNA genetic markers we managed to identify 32 vicims of Konfin I mass grave with the PP higher than 99,9 %
- The skeletal remains were returned to the living relatives for funeral

V petek, 18. julija 2011, so v Veliki Ligojni pokopali brata Janeza in Jakoba Borštnika ter Jožeta Verbiča. Vsi trije so bili umorjeni 24. junija 1945 v breznu pri Konfinu I.

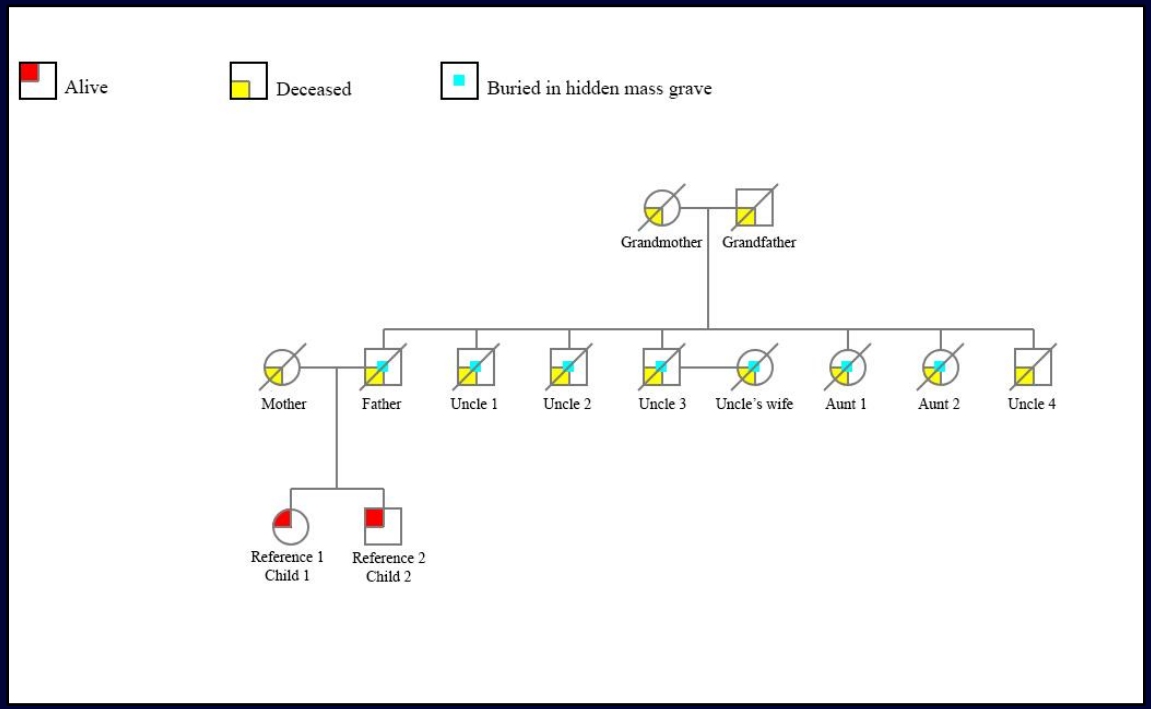
Int J Legal Med (2010) 124:307–317
DOI 10.1007/s00414-010-0431-y

ORIGINAL ARTICLE

Molecular genetic identification of skeletal remains from the Second World War Konfin I mass grave in Slovenia

Irena Zupanič Pajnič · Barbara Gornjak Pogorelc · Jože Balažic

Identification of the victims of the biggest WWII family killing in Slovenia (Babna gora mass grave)



- 10 members of the same family were killed in 1942, and seven of them were buried in hidden mass grave. This seven victims of the same family were father, three uncles, two aunts and a wife of the oldest uncle.
- Family references (son and daughter).

Identification of the victims of the biggest WWII family killing in Slovenia (Babna gora mass grave)

In March 2015, the excavation of the remains began, but only 3 incomplete female skeletons were excavated.

Identification of the victims of the biggest WWII family killing in Slovenia (Babna gora mass grave)

- Only 20 meters away, relatives encountered bones later, and in Avgust 2016, a burial site of at least 3 males was excavated. The vicitms were buried in the forest in shallow graves and the excavated skeletons were incomplete in both graves.
- A total of 12 bones and teeth were analysed and compared to two living relatives (son and daughter).
- We analysed the left second molar, femur and tibia from one female skeleton and femur and tibias from another two victims from the female grave. From the male grave 6 femurs were analysed.
- we managed to obtain nuclear DNA for successful STR typing from 7 bones and one molar. From the female grave profiles were obtained only for one victim (identical profiles of left second molar, femur and tibia) and from the male grave from five femurs (among them there were two pairs of femurs).

Female grave

- From badly preserved incomplete skeletal remains genetic typing was successful only for one skeleton out of three
- Two bones (femur and tibia) and the left second molar were analysed.

bone/tooth sample	Autosomal target (Auto)	Degradation target (Deg)	Y target (Y)	IPC Shift	[Auto]/[Deg] ratio
female grave-SKEL. 1-FEMUR	0.0003	/	/	0.59	undetermined
female grave-SKEL. 1-TIBIA	/	/	/	-0.49	/
female grave-SKEL. 2-LM2	0.0054	0.0012	/	-0.44	4.61
female grave-SKEL. 2-FEMUR	0.0029	/	/	-0.07	undetermined
female grave-SKEL. 2-TIBIA	0.0020	0.0006	/	-0.38	3.22
female grave-SKEL. 3-TIBIA	/	/	/	-0.31	/
female grave-ENC	/	/	/	-0.33	/

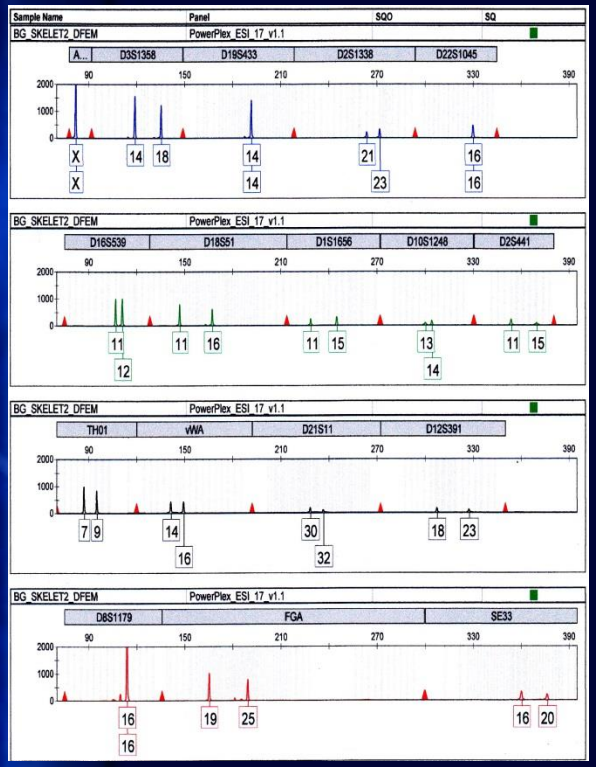
Femur

LM2

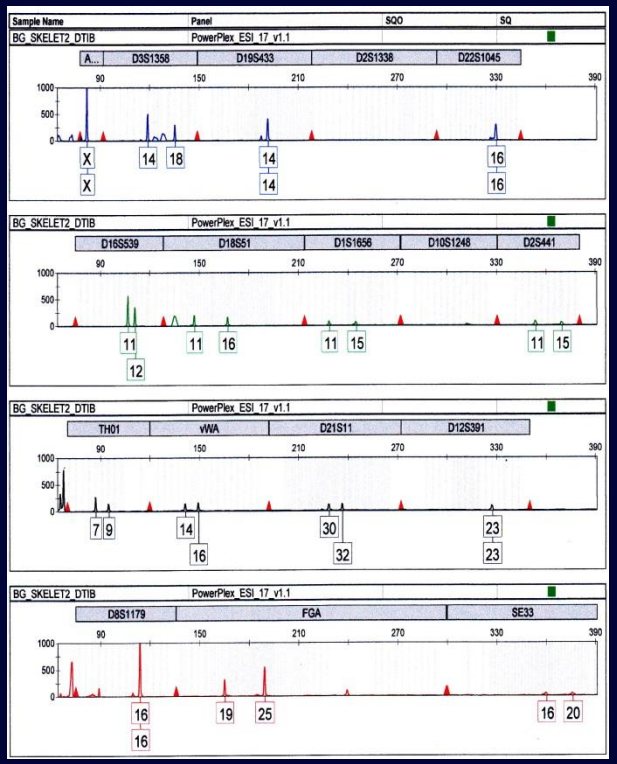
Tibia

Autosomal genetic profiles of identified aunt

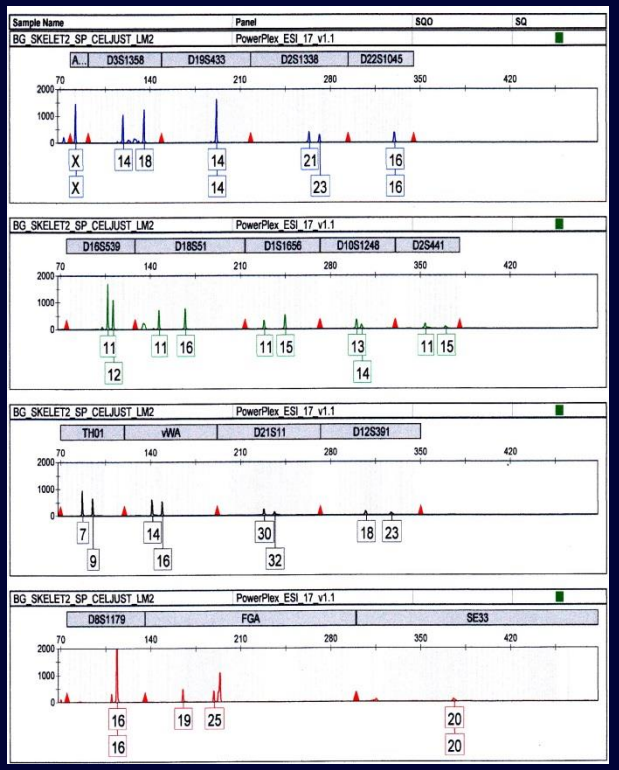
Femur



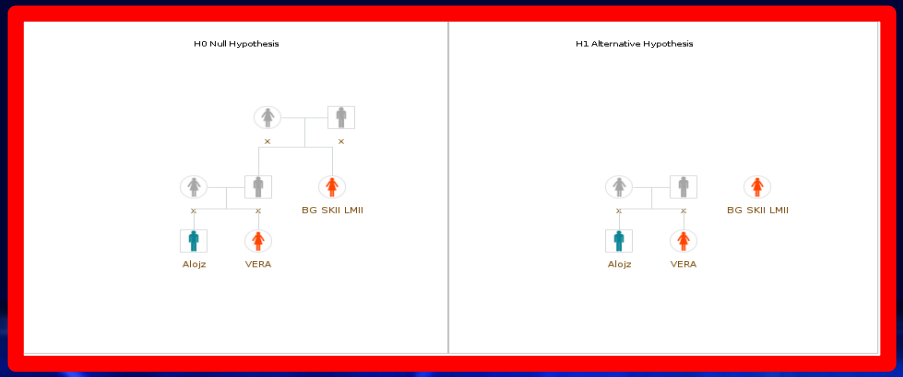
Tibia



LM2

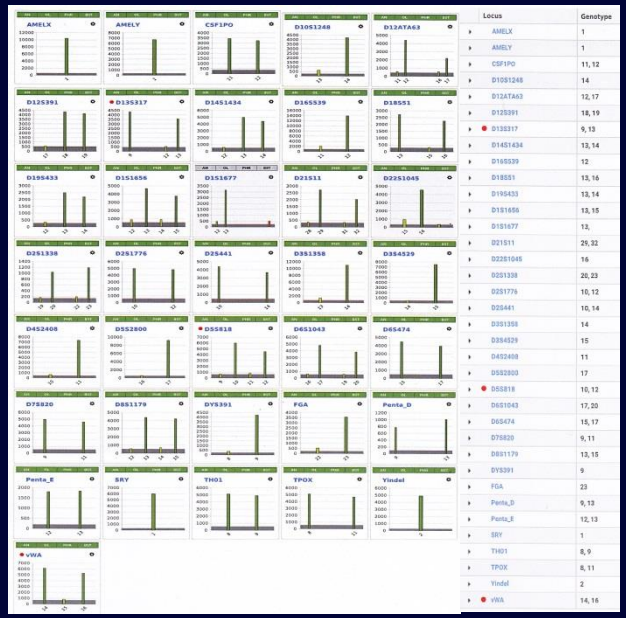


After comparison to the nephew and niece LR was calculated for autosomal STRs and too low PP was obtained.

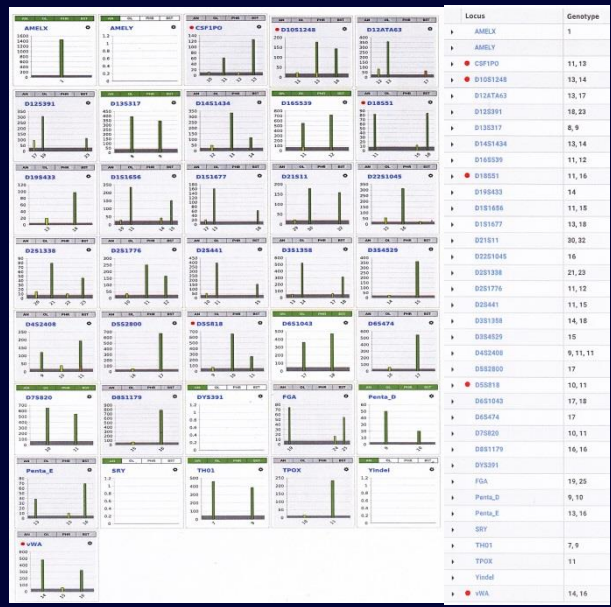


Autosomal genetic profiles of the niece, nephew and the tooth LM2 obtained with the Precision ID GlobalFiler NGS STR Panel (TFS)

Niece



Nephew



LM2

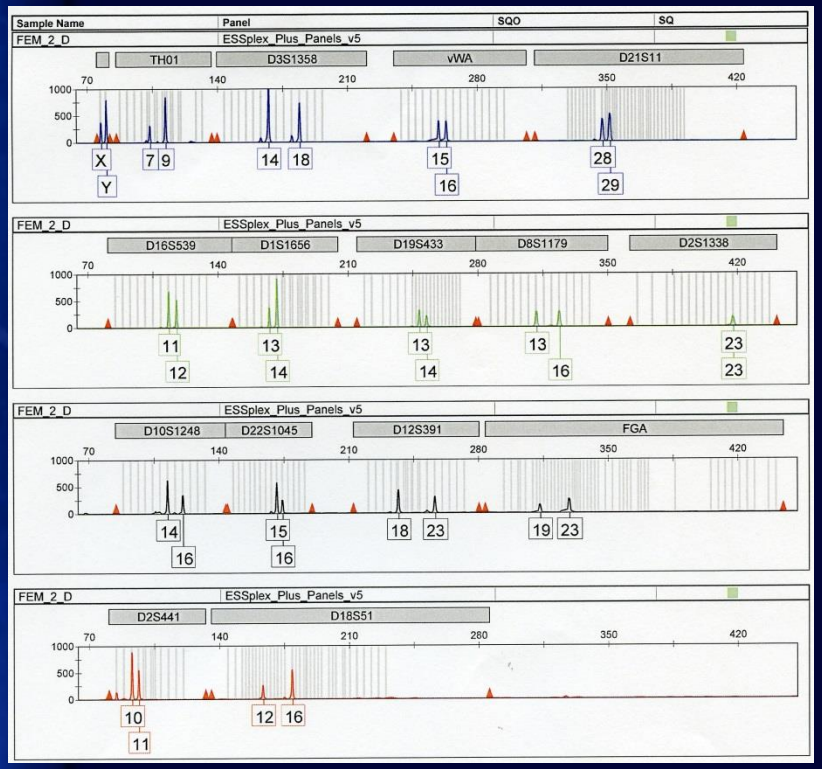
Since PP of 99.9% was followed, the massive parallel sequencing Precision ID GlobalFiler NGS STR Panel (TFS) was used and after the analysis of additional STR loci (31 STRs) the statistical calculation showed the PP of 99.99986% indicating that a sufficient number of genetic markers were investigated in identifying skeletal remains of the aunt.

Male grave

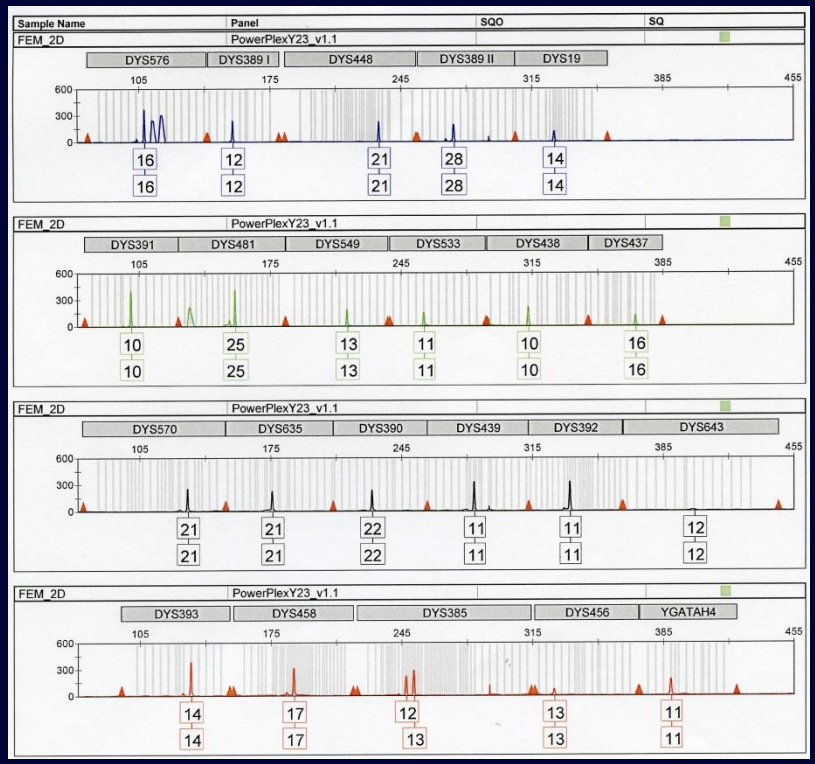
- From the male grave profiles were obtained from five femurs out of six (among them there were two pairs of femurs).
- Full autosomal profiles allowing the identification of 3 male relatives - two uncles and the father of two cildren used as a family references.
- The relationships between males (father/son, uncles/nephew and brother vicitms) were further confirmed by the analyses of Y-STRs.
- The product rule was used to estimate a combined LR for autosomal and Y-STRs and the statystical analyses showed a high confidence of correct identification with posterior probability higher than 99,9% for all three male victims identified.

bone/tooth sample	Autosomal target (Auto)	Degradation target (Deg)	Y target (Y)	IPC Shift	[Auto]/[Deg] ratio
male grave-FEMUR 1	0.0270	0.0060	0.0149	-0.53	4.47
male grave-FEMUR 2	0.0046	0.0018	0.0031	-0.44	2.57
male grave-FEMUR 3	0.0003	/	0.0002	-0.42	undetermined
male grave-FEMUR 4	0.0015	0.0002	0.0005	-0.51	7.38
male grave-FEMUR 5	0.0048	0.0009	0.0017	-0.51	5.12
male grave-FEMUR 6	0.0193	0.0059	0.0107	-0.42	3.29
male grave-ENC	/	/	/	-0.24	/

Autosomal and Y-STR genetic profiles of identified father

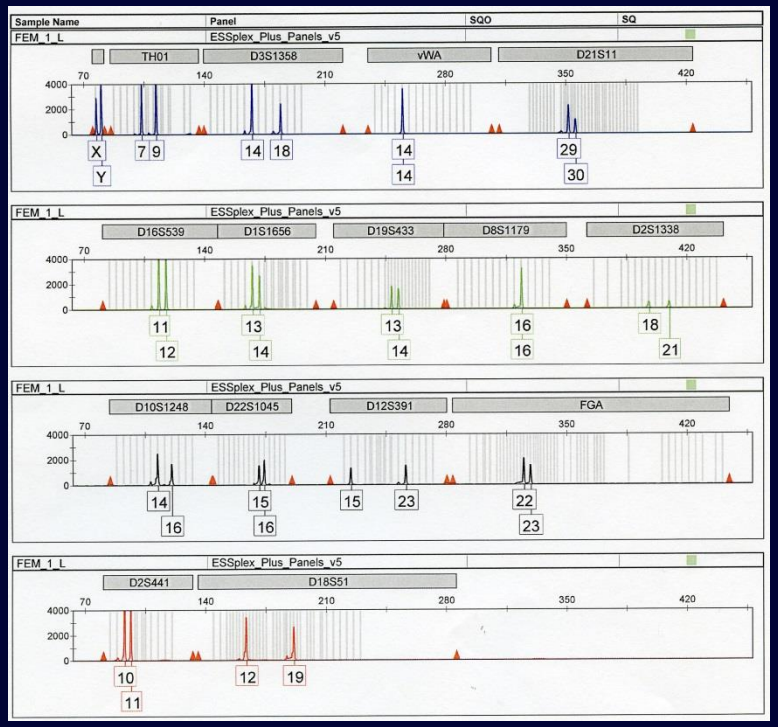


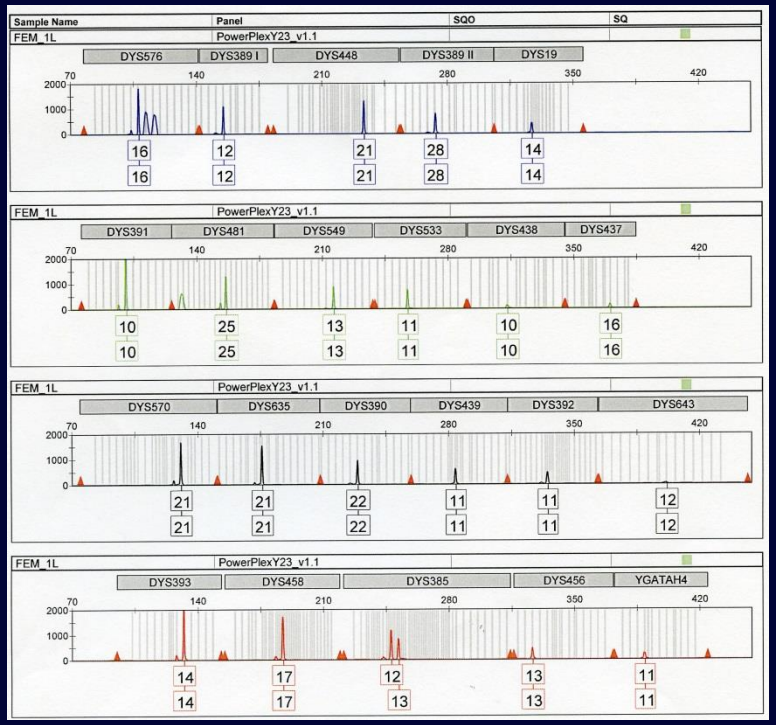
Autosomal STR profile



Y-STR haplotype

Autosomal and Y-STR genetic profiles of identified uncle 1

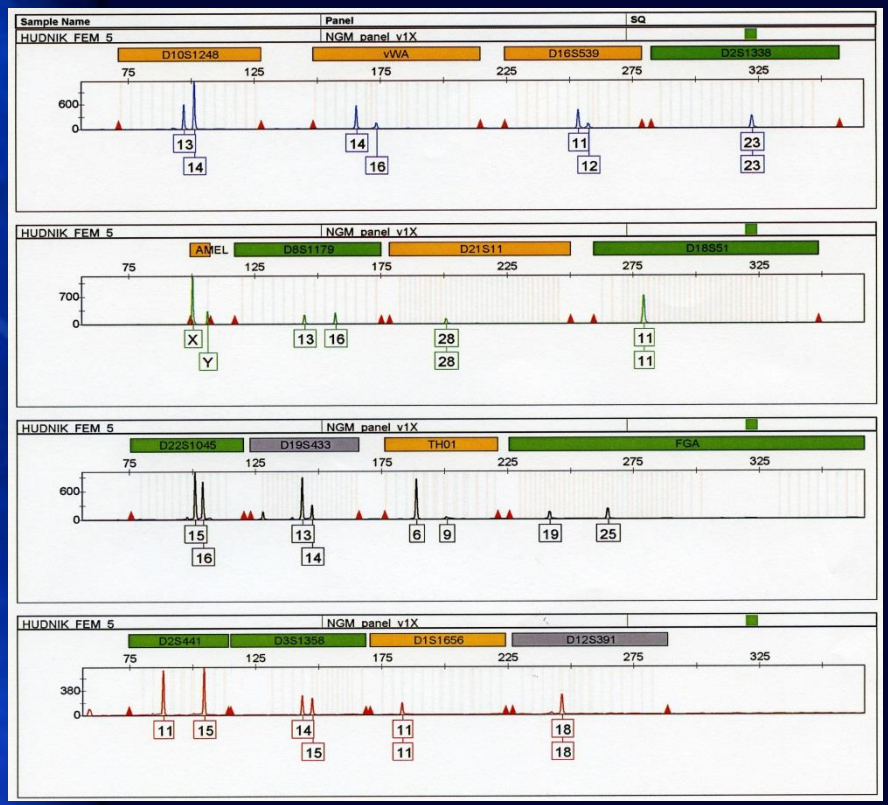


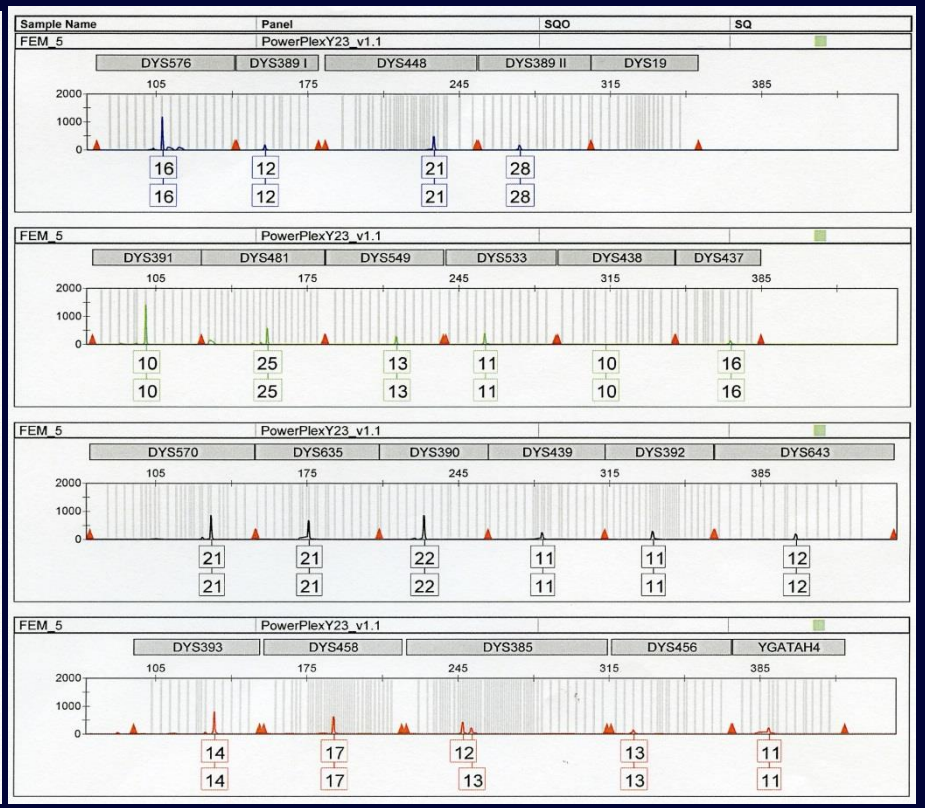


Autosomal STR profile

Y-STR haplotype

Autosomal and Y-STR genetic profiles of identified uncle 2





Autosomal STR profile

Y-STR haplotype

Identification of the victims of the biggest WWII family killing in Slovenia (Babna gora mass grave)

	Aunt (skeleton 2- female grave)	Father (femur 2-male grave)	Uncle 1 (femur 1 and femur 6-male grave)	Uncle 2 (femur 4 and femur 5-male grave)
Reference	nephew and niece	son and daughter	nephew and niece	nephew and niece
No. n-STRs typed	31	15	15	15
No. Y-STRs typed		23	23	23
LR $_{(n\text{-}STR)}$	4.9×10^6	2.1×10^9	8.6×10^7	5.3×10^6
PP $_{(n\text{-}STR)}$	99.99986%	99.9999997%	99.999992%	99.9999%
LR $_{(Y\text{-}STR)}$		2	2	2
LR $_{(n\text{-}STR \times Y\text{-}STR)}$		4.3×10^9	1.7×10^8	1.1×10^7
PP $_{(n\text{-}STR \times Y\text{-}STR)}$		99.9999998%	99.999996%	99.99993%

- Full autosomal and Y-STR profiles allowing the identification of 4 family members:
  - one of the aunt from the female grave and
  - two uncles and the father of two cildren used as a family references from the male grave.
- For traceability in the event of contamination, we created an elimination database (profiles of individuals that participated in the excavation, anthropological investigation and genetic analyses) and no match was found.
- After more than 70 years, the skeletal reamins were returned to the surviving children (brother and sister) who buried their relatives in a family tomb.

Identification of skeletal remains of the spouse Hribar

The spouse Hribar were liquidated in January 1944 near their castle (Strmol). They came from well-known and wealthy Slovenian families, who were part of the pre-war elite in Slovenia. Rado was a banker and an industrialist, Ksenija was the first Yugoslav pilot.

Identification of skeletal remains of the spouse Hribar

The hidden grave with skeletal remains of the spouse Hribar was found in 2015 and only incomplete remains of a male and female skeleton were excavated.

The living relatives were traced only for Rado (two paternal nephews and niece) and since spouses did not have children the genetic identification of Ksenija was not possible.

Identification of skeletal remains of the spouse Hribar

Skeleton A – Rado Hribar

Autosomal and Y-STR typing of:

1. Femur
2. Left second molar
3. Tibia

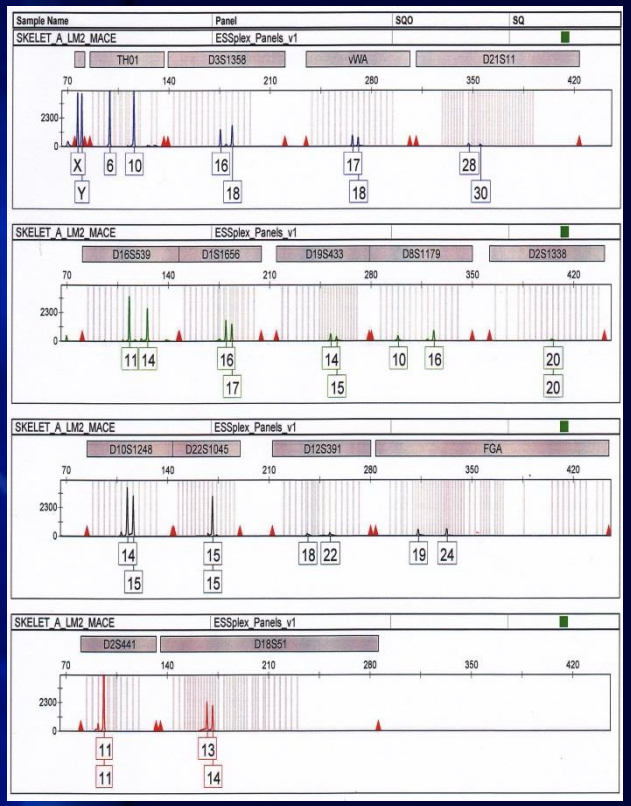
Femur

LM2

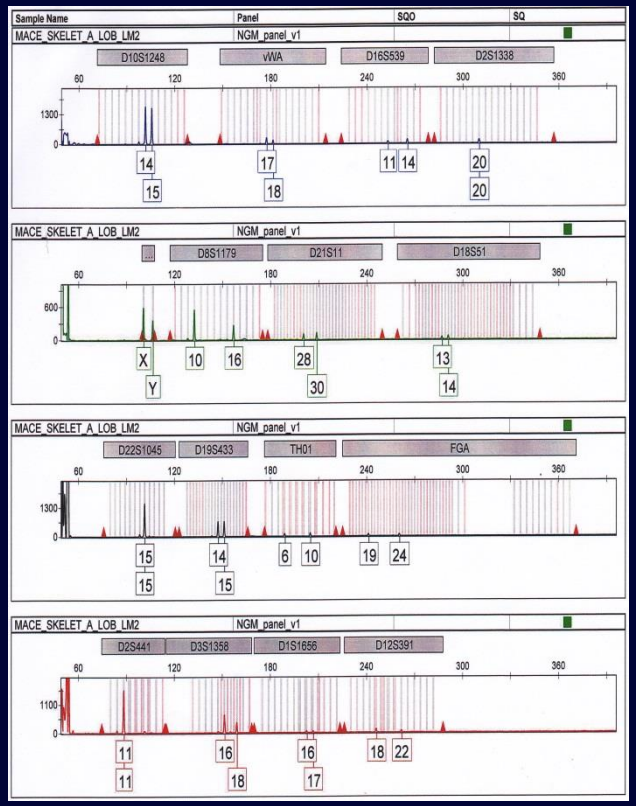
Tibia

Skeleton A (LM2) – Rado Hribar

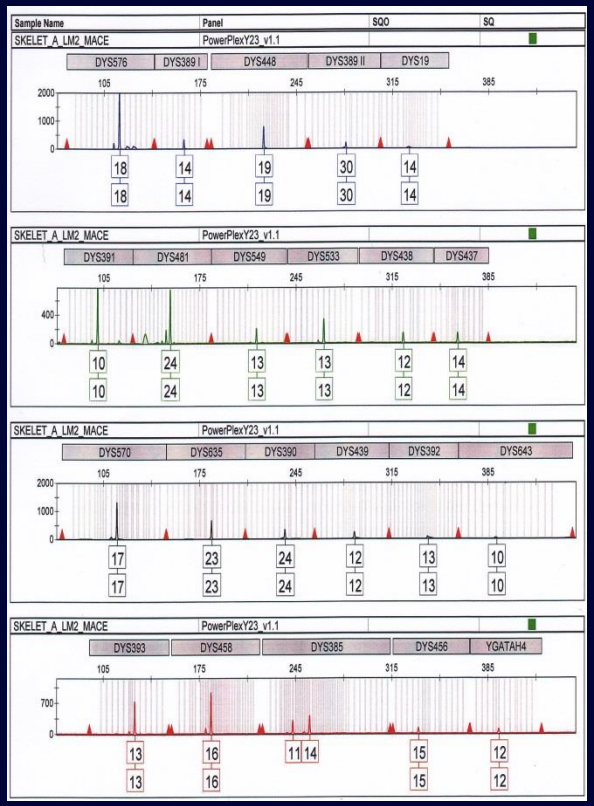
ESSPlex Plus kit



NGM kit



PowerPlex Y23 kit

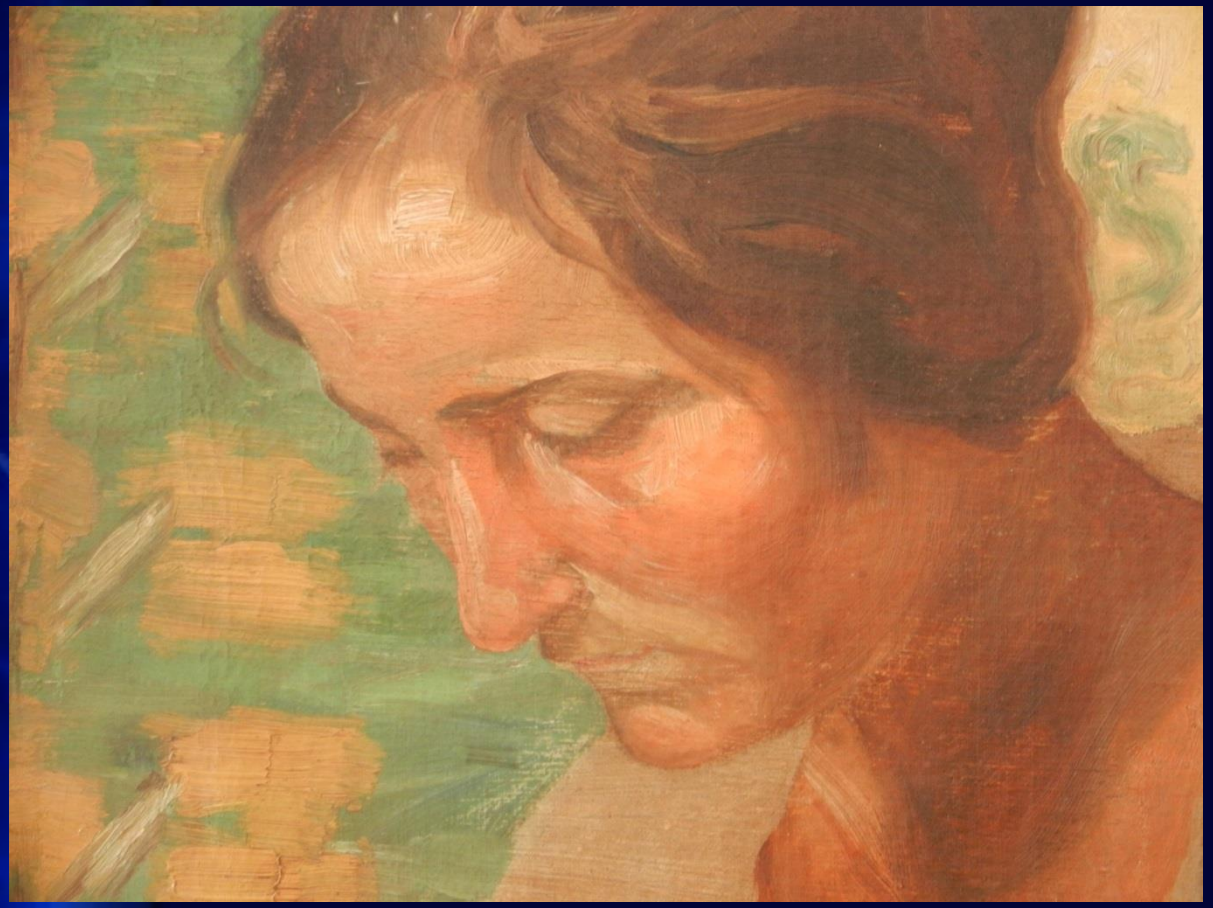


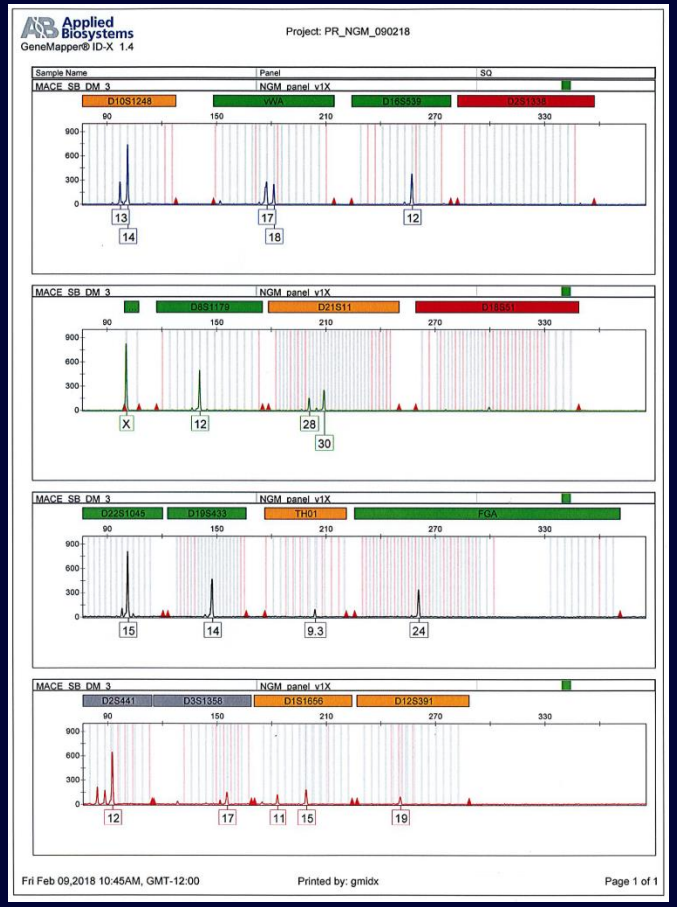
Full autosomal and Y-STR profiles allowing the identification of the Slovenian castle man Rado Hribar by comparison with family references and the relationships between males (uncle and nephews) were confirmed by Y-STRs.

Skeleton B – Ksenija Hribar

Autosomal STR typing of right third molar

Skeleton B – Ksenija Hribar





- Autosomal STR profile – gender identification (female)
- Phenotypic SNPs – hair and eye colour

Identified victims of Slovenian mass graves

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Research Paper

Searching for the mother missed since the Second World War

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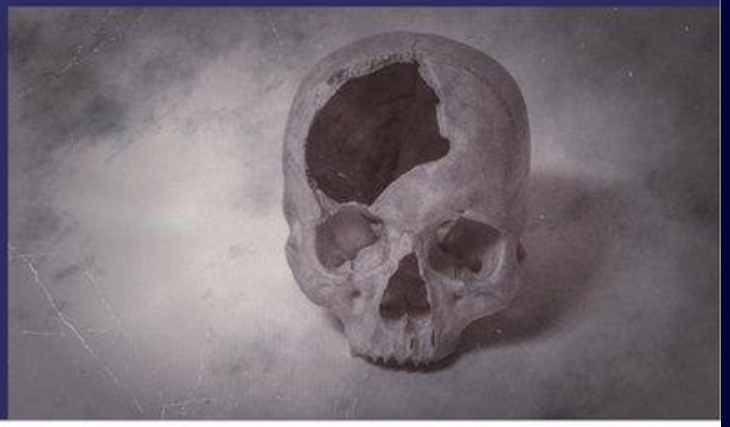
ORIGINAL ARTICLE

Molecular genetic identification of skeletal remains from the Second World War Konfin I mass grave in Slovenia

Irena Zupanič Pajnič · Barbara Gornjak Pogorelc · Jože Balažic

Monograph

This book describes genetics investigations of DNA from a Second World War victim's skeletal remains and their genetic identification. The characteristics of ancient DNA and the environmental factors that affect its preservation are described. The recommendations for excavation, storage and molecular genetic identification of skeletal remains are presented. The method of how reference samples from relatives and samples for the elimination database are collected and analysed for each mass grave is shown. The most appropriate types of bones and teeth for genetic analyses are described and the measures for preventing contamination in the DNA laboratory are listed. Procedures for processing the bone sample (mechanical and chemical cleaning, cutting and grinding into powder), decalcification of bone powder, DNA extraction, DNA quantification and DNA typing of autosomal and Y chromosomal microsatellites and mitochondrial DNA are described and interpretation of genetic profiles with statistical calculations of the likelihood ratio and posterior probability are exposed. Some examples of identification of the Second World War mass grave victims are presented.



Irena Zupanič Pajnič

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978-3-659-45306-9

Irena Zupanič Pajnič

Genetic Identification of Second World War Victim's Skeletal Remains

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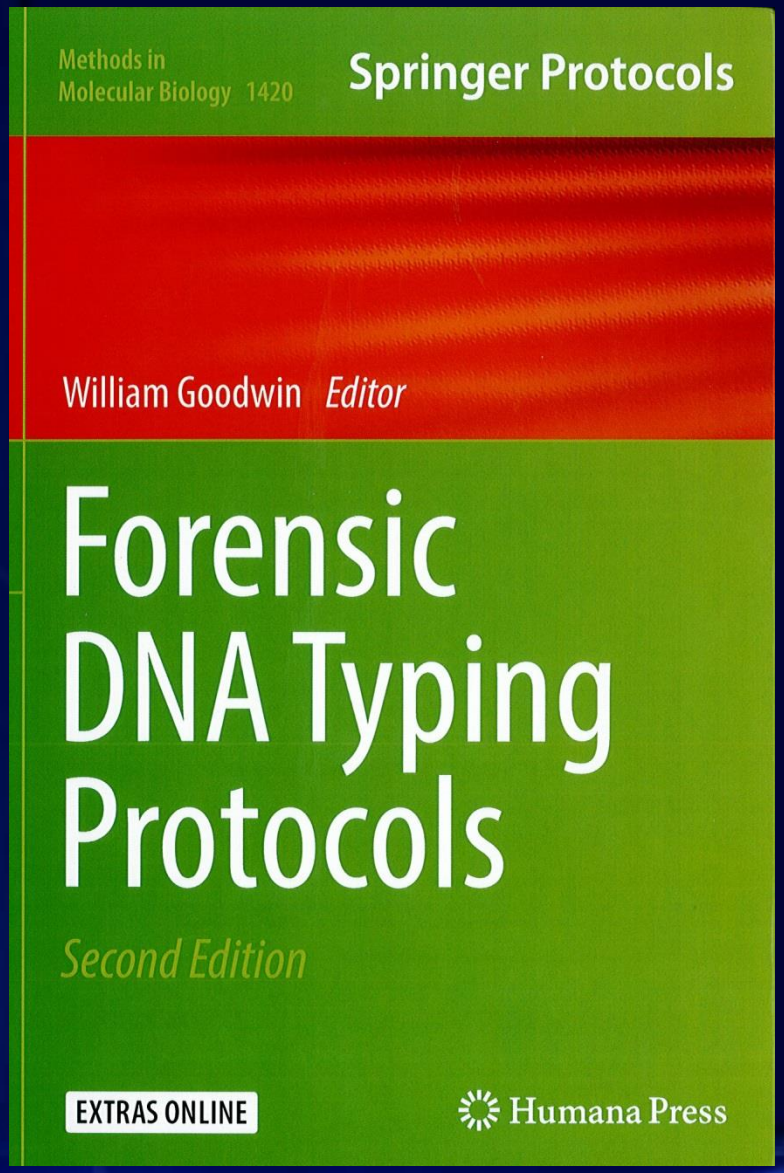
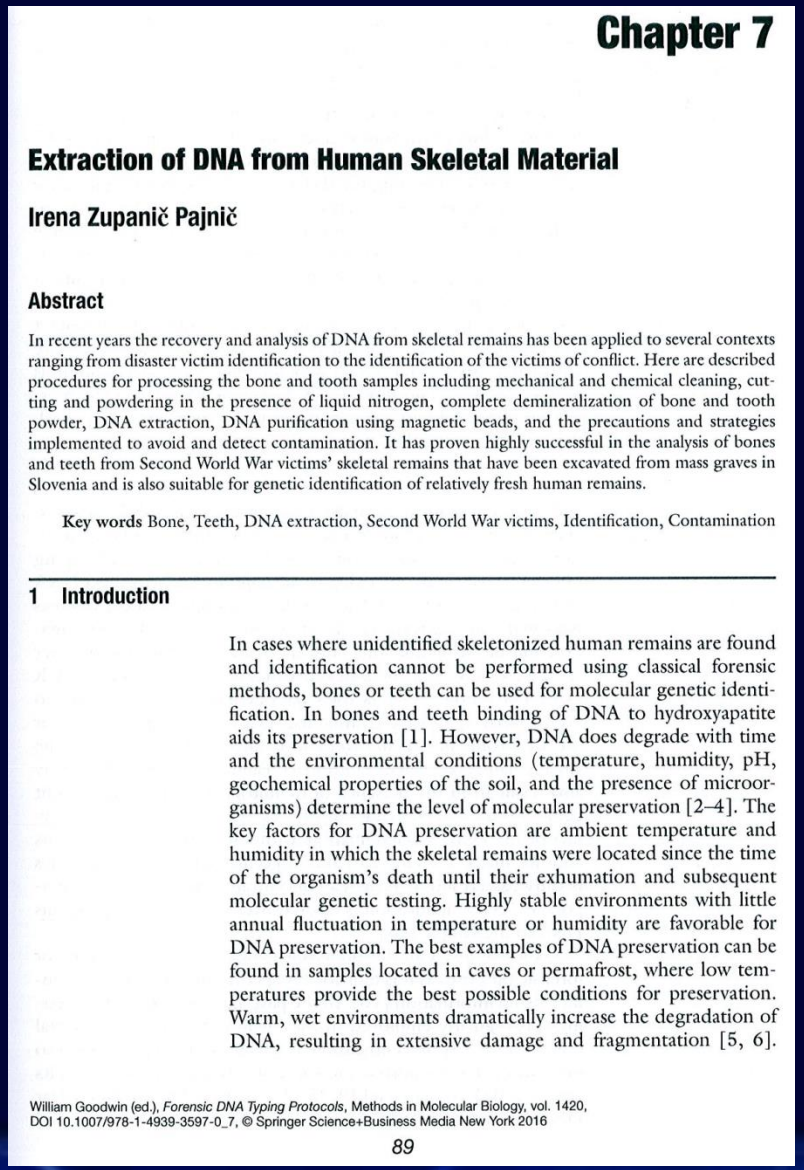
Chapter in Monograph





Chapter 7

Extraction of DNA from Human Skeletal Material

Irena Zupanič Pajnič

Abstract

In recent years the recovery and analysis of DNA from skeletal remains has been applied to several contexts ranging from disaster victim identification to the identification of the victims of conflict. Here are described procedures for processing the bone and tooth samples including mechanical and chemical cleaning, cutting and powdering in the presence of liquid nitrogen, complete demineralization of bone and tooth powder, DNA extraction, DNA purification using magnetic beads, and the precautions and strategies implemented to avoid and detect contamination. It has proven highly successful in the analysis of bones and teeth from Second World War victims' skeletal remains that have been excavated from mass graves in Slovenia and is also suitable for genetic identification of relatively fresh human remains.

Key words Bone, Teeth, DNA extraction, Second World War victims, Identification, Contamination

1 Introduction

In cases where unidentified skeletonized human remains are found and identification cannot be performed using classical forensic methods, bones or teeth can be used for molecular genetic identification. In bones and teeth binding of DNA to hydroxyapatite aids its preservation [1]. However, DNA does degrade with time and the environmental conditions (temperature, humidity, pH, geochemical properties of the soil, and the presence of microorganisms) determine the level of molecular preservation [2–4]. The key factors for DNA preservation are ambient temperature and humidity in which the skeletal remains were located since the time of the organism's death until their exhumation and subsequent molecular genetic testing. Highly stable environments with little annual fluctuation in temperature or humidity are favorable for DNA preservation. The best examples of DNA preservation can be found in samples located in caves or permafrost, where low temperatures provide the best possible conditions for preservation. Warm, wet environments dramatically increase the degradation of DNA, resulting in extensive damage and fragmentation [5, 6].

William Goodwin (ed.), *Forensic DNA Typing Protocols*, Methods in Molecular Biology, vol. 1420,
DOI 10.1007/978-1-4939-3597-0_7,

Research work on victims of Slovenian mass graves

Forensic Science International: Genetics 26 (2017) 48–57

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Bringing colour back after 70 years: Predicting eye and hair colour from skeletal remains of World War II victims using the HIrisPlex system

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DOI 10.1007/s00414-017-1600-z

SHORT COMMUNICATION

Rapidly mutating Y-STR analyses of compromised forensic samples

Rashed Alghafri[1] · Irena Zupanič Pajnič[2] · Tomaž Zupanc[2] · Jože Balažic[2] · Pankaj Shrivastava[3]

Forensic Science International: Genetics 27 (2017) 17–26

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Prediction of autosomal STR typing success in ancient and Second World War bone samples

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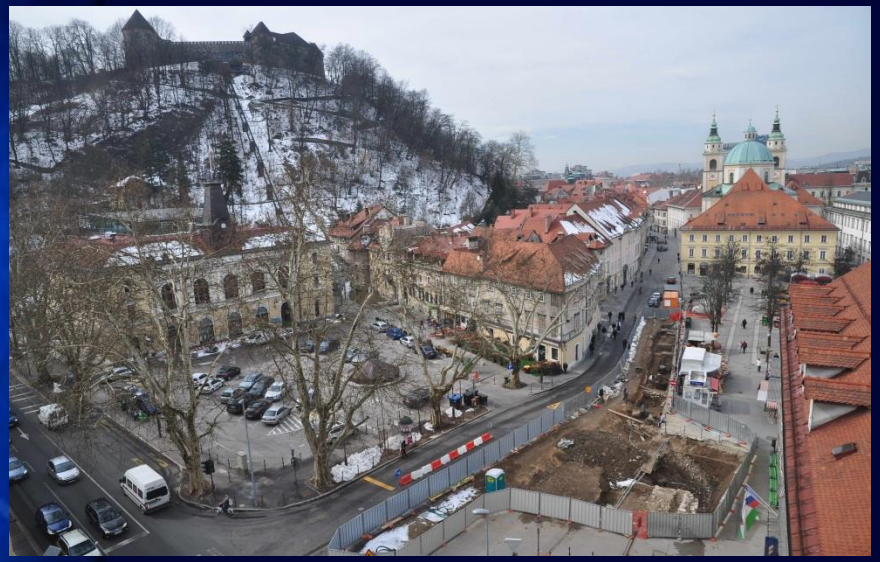
ANCIENT SKELETONS
AUERSPERG family

- The Auersperg (Turjaški) were the most influential aristocrat family on Slovenian territory and one of the richest in Hapsburg Empire. They settled Kranjska in 11th century and left Slovenia before World War II. In 2009 the archaeologists excavated five skeletons from the 17th century archaeological site in the centre of Ljubljana (at Market place). In 2011 we have been asked for their identification.

Auersperg chapel archaeological site



- The genetic typing of five skeletons from Auersperg chapel archaeological site presents the first archaeogenetic research in Slovenia. Skeletons were found in the side chapel of the church in the Franciscans monastery which was the Auersperg tomb.

Auersperg chapel archaeological site

- Beside the skeletons the bronze bowl with the heart was found and the name of Ferdinand II and the year of death (1655 - 1706) engraved.

Condition of skeleton 1 after the excavation

No femurs and no teeth

Skeleton 1

Condition of skeleton 2 after the excavation

No femurs and no teeth

Skeleton 2

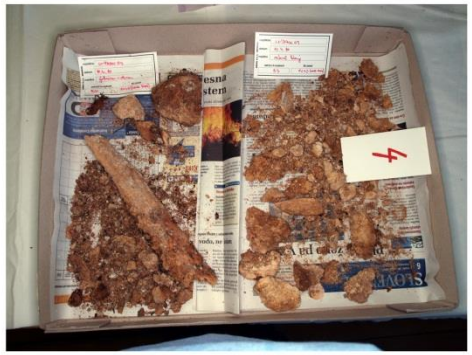
Condition of skeleton 3 after the excavation

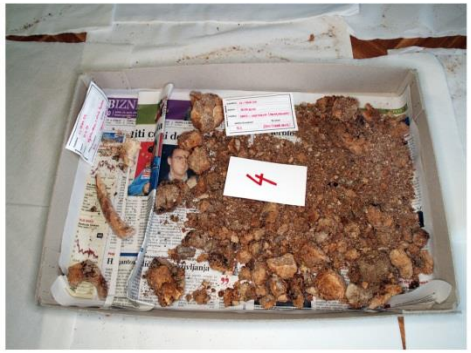
No femurs and no teeth

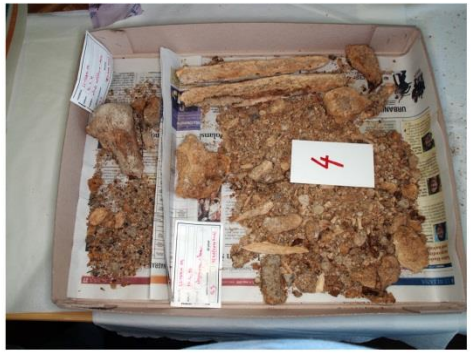
Skeleton 3

Condition of skeleton 4 after the excavation










Fragments of femurs were preserved

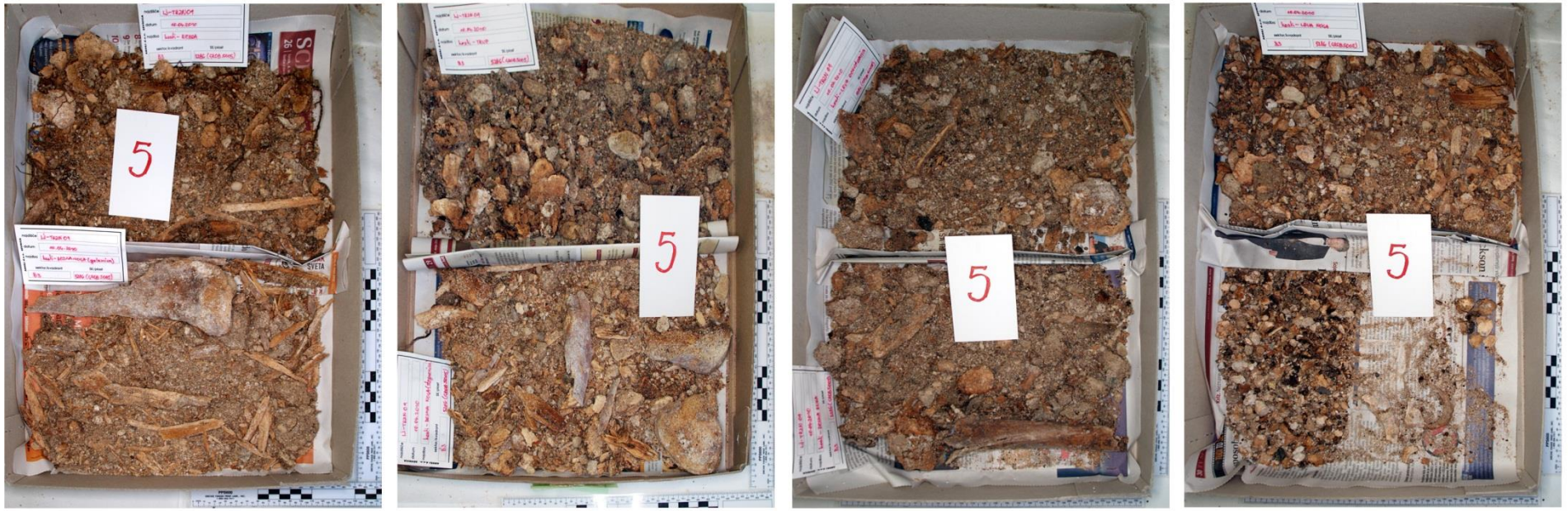
Skeleton 4

Condition of skeleton 4 after the excavation

Teeth were preserved

Skeleton 4

Condition of skeleton 5 after the excavation



Fragments of femurs were preserved

Skeleton 5

Condition of skeleton 5 after the excavation

Teeth were preserved

Skeleton 5

Selection of bones and teeth for DNA analyses

Skeleton 1 (cranium: os frontale)

Skeleton 2
(cranium: os frontale, parietale)

Skeleton 3 (cranium: os occipitale)

Skeleton 4 (femurs)

Skeleton 4 (maxilla)

Skeleton 4 (mandibula)

Skeleton 4 (mandibula LM2, maxilla RM3, LM3 and RM2)

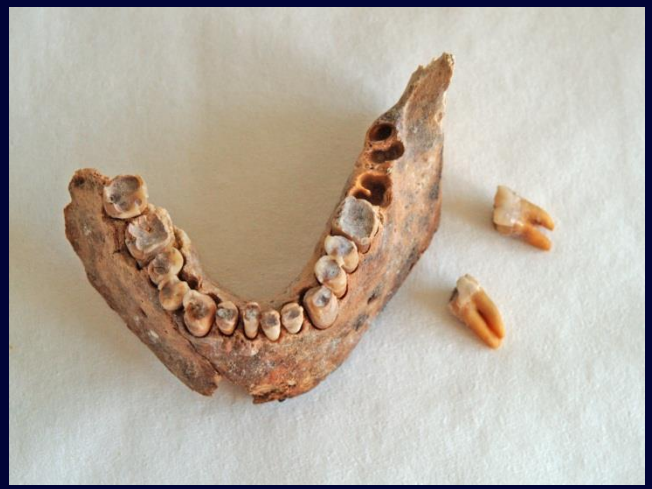
Selection of bones and teeth for DNA analyses

Skeleton 5 (femur)

Skeleton 5 (maxilla)

Skeleton 5 (mandibula)

Skeleton 5 (maxilla RM2, LM3)



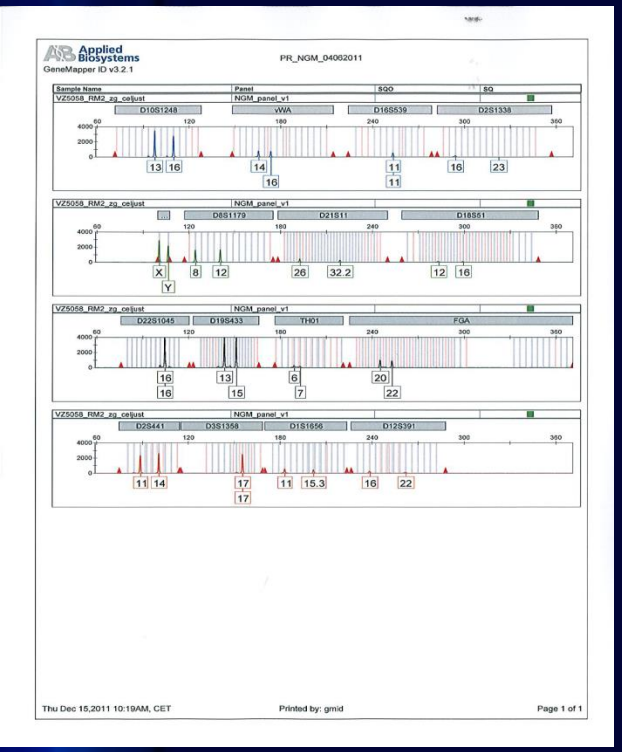
Skeleton 5 (mandibula LM2, LM3)

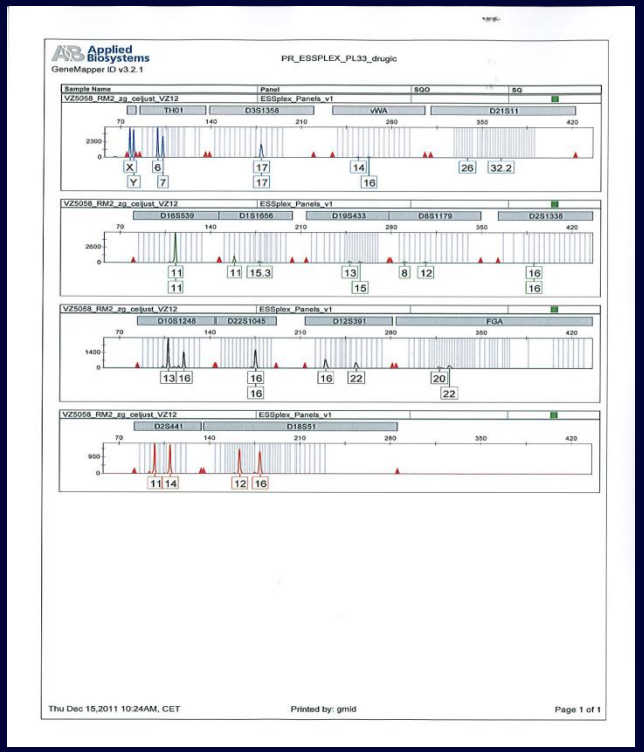
Condition of the heart from the bronze bowl after the excavation

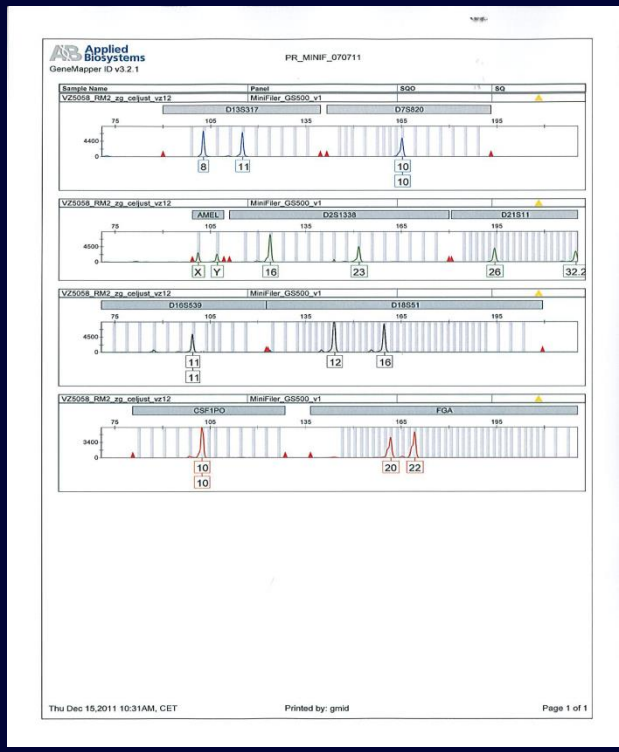
Sampling of the heart from the bronze bowl for DNA analyses

1. Inner layer
2. Outer layer

Autosomal genetic profiles of skeleton 4 (teeth)



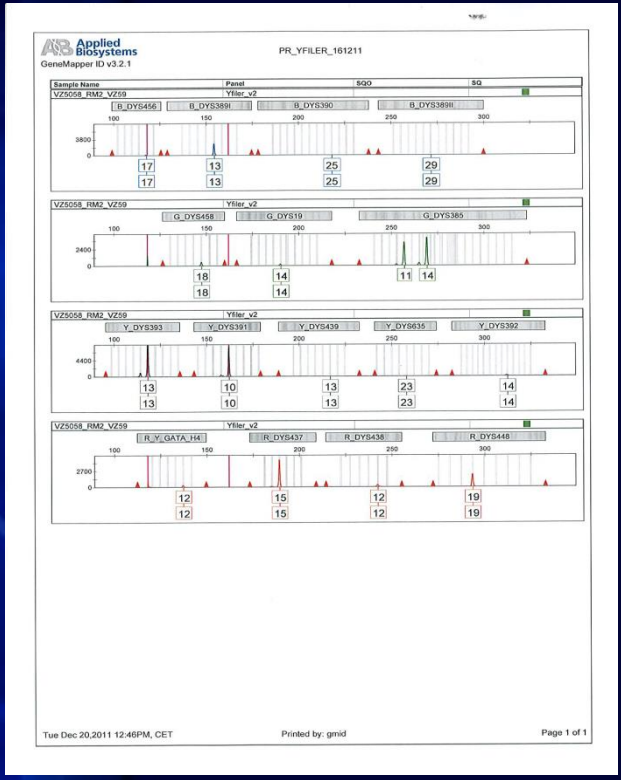


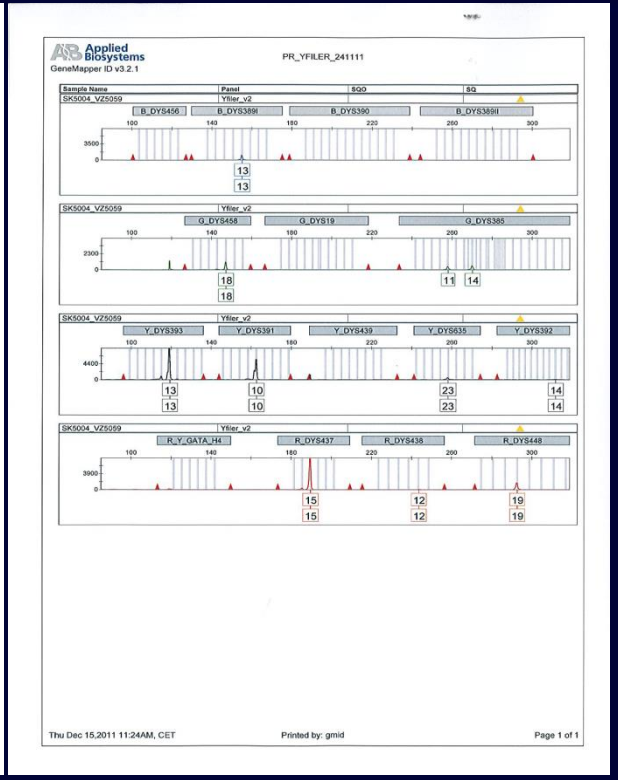


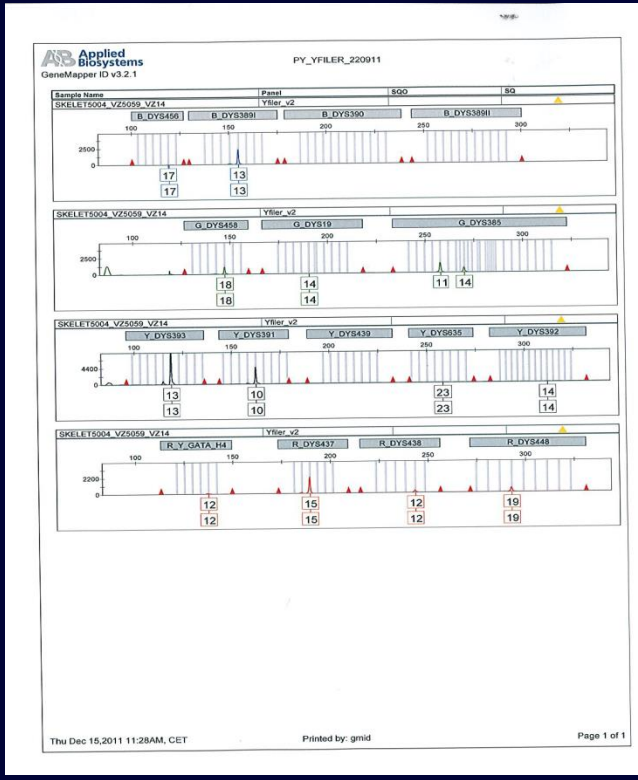
- Male genetic profile

Vzorec	D10	vWA	D16	D2S1	D8S	D21	D18	D22	D19	
Sk.5004	13/16	14/16	11/11	16/23	8/12	26/32.2	12/16	16/16	13/15	
Vzorec	**THO1**	**FGA**	**D2S4**	**D3**	**D1**	**D12**	**D13**	**D7**	**CSF1PO**	**Amelo.**
Sk.5004	6/7	20/22	11/14	17/17	11/15.3	16/22	8/11	10/10	10/10	X/Y

Y-STR genetic profile of skeleton 4 (teeth)



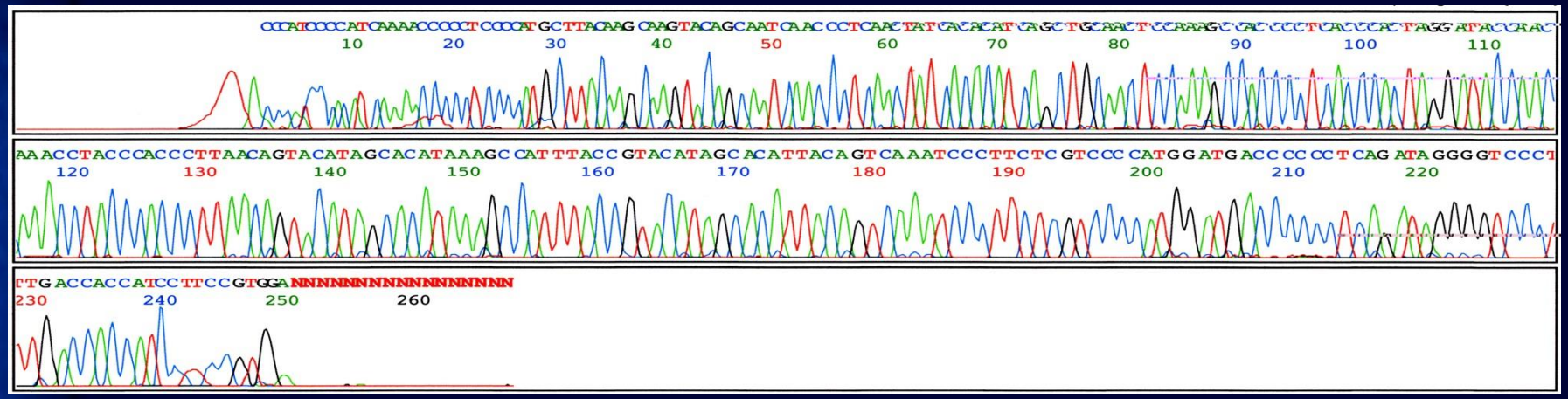




- Almost complete Y-STR haplotype (to track the paternal line)

Vzorec	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385a/b	DYS393
Sk.5004	17	13			18	14	11/14	13
Vzorec	**DYS391**	**DYS439**	**DYS635**	**DYS392**	**DYSH4**	**DYS437**	**DYS438**	**DYS448**
Sk.5004	10		23	14	12	15	12	19

MtDNA sequence of skeleton 4 (teeth)



- We obtained mtDNA haplotype from skeleton 4 (teeth) (to track the maternal line)

Sk. 5004	HVI: identical CRS HVII: 263(G), 309.1(C), 315.1(C)	HVI: 16030-16381 HVII: 55-388

Elimination database: DNA typing of autosomal and Y-STRs

- For traceability in the event of contamination, we created an elimination database including genetic profiles of the nuclear and mtDNA of all persons that had been in contact with the skeletal remains and no match was found.

Vzorec	D10S1248	vWA	D16S539	D2S1338	Amelog.	D8S1179	D21S11	D18S51
I. Z. P. (ISM)	15/16	17/18	11/12	24/26	X/X	13/15	30/32.2	12/16
B. G. P. (ISM)	14/16	14/16	9/9	17/20	X/X	10/14	29/31	10/17
K. V. M. (ISM)	14/14	18/18	9/12	17/25	X/X	13/16	28/30	17/19
B. E. (ISM)	13/15	14/19	9/13	19/24	X/Y	13/14	29/33.2	12/14
K. P. (ISM)	13/14	14/18	12/13	23/24	X/X	13/13	30/32.2	11/15
K. I. (ISM)		14/17	12/12	20/24	X/X	12/13	30/31.2	14/17
R. B. (ISM)		14/19	10/12	17/21	X/Y	13/14	30/32.2	14/19
S. P. (arheolog)	14/14	16/18	11/11	20/23	X/Y	14/16	29/30	12/13
M. B. (arheolog)	14/14	14/17	11/12	17/20	X/Y	13/13	31/32	12/14
M. D. (arheolog)	14/15	16/19	10/12	17/25	X/Y	13/15	30/30	14/14
R. M. (arheolog)	13/16	14/18	10/12	17/19	X/Y	10/12	29/29	13/16
T. K. (arheolog)	15/16	17/18	9/13	18/19	X/Y	13/15	29/30	14/14
M. L. (arheolog)	13/14	14/17	11/13	17/17	X/Y	10/11	30/31.2	16/17
P. P. (arheolog)	13/14	15/16	11/13	17/24	X/Y	12/15	29/29	12/15
I. B. Z. (arheolog)	13/14	17/17	12/12	23/24	X/X	13/13	31.2/31.2	15/16
D. A. (arheolog)	14/16	16/18	9/12	20/23	X/X	14/14	29/30	17/19
T. L. (arheolog)	13/15	17/17	10/12	18/19	X/X	8/12	28/30	16/19
T. T. R. (antropolog)	12/16	17/17	11/12	19/20	X/X	13/15	28/32.2	15/16
	D22S1045	D19S433	THO1	FGA	D2S441	D3S1358	D1S1656	D12S391
I. Z. P. (ISM)	15/15	15/16	6/9	22/24	10/11.3	14/18	11/14	17/18
B. G. P. (ISM)	16/16	13/15.2	7/9.3	20/20	14/14	14/17	12/17.3	22/22
K. V. M. (ISM)	16/16	13/14	8/9	19/20	10/11	16/16	11/11	15/21
B. E. (ISM)	11/15	13/15	7/8	22/24	11/11	16/17	14/15	21/24
K. P. (ISM)	11/11	12/13	6/9	20/24	14/15	14/17	12/15	17/20
K. I. (ISM)		13/13	8/9.3	22/24		18/18		
R. B. (ISM)		14/14	6/9.3	21/24		15/16		
S. P. (arheolog)	11/16	13/14	9.3/9.3	21/22	11.3/13	16/18	15/19.3	17/22
M. B. (arheolog)	11/15	13/13	6/6	21/23	11/14	14/16	11/13	18/20
M. D. (arheolog)	11/16	13/14	7/9.3	21/22	11.3/14	15/16	11/15.3	15/21
R. M. (arheolog)	11/15	13/13	9/9.3	19/23	10/11	14/17	15.3/17	15/19
T. K. (arheolog)	16/16	14/15	6/8	20/25	14/14	15/17	12/16.3	18/22
M. L. (arheolog)	14/16	13/16	9/9.3	22/24	10/14	15/17	16.3/17.3	20/21
P. P. (arheolog)	15/16	13.2/15	6/8	19/21	10/11	16/17	11/17	17/25
I. B. Z. (arheolog)	16/16	13/13	6/7	20/20	14/14	16/17	11/12	17/18
D. A. (arheolog)	11/16	12/16	9/9.3	18/19	10/14	18/18	13/14	18/25
T. L. (arheolog)	15/16	13/14	8/9.3	20/22	11/14	14/17	11/15	21/24
T. T. R. (antropolog)	11/16	15/15	6/9.3	23/24	11/14	15/17	17.3/18.3	19/20

Vzorec	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385a/b	DYS393
B. E. (ISM)	17	14	24	31	15	17	11/14	13
R. B. (ISM)	15	13	24	30	16	17	14/15	13
S. P. (arheolog)	15	14	22	30	17	14	14/17	12
M. B. (arheolog)	15	13	24	32	17	16	14/15	13
M. D. (arheolog)	17	13	25	30	15	16	12/14	13
R. M. (arheolog)	16	13	27	29	16	17	10/14	13
T. K. (arheolog)	15	13	25	31	15	16	11/14	13
M. L. (arheolog)	15	13	24	29	15	14	11/11	13
P. P. (arheolog)	15	13	25	29	17	14	11/14	13
	DYS391	DYS439	DYS635	DYS392	DYSH4	DYS437	DYS438	DYS448
B. E. (ISM)	11	10	23	11	12	14	11	20
R. B. (ISM)	11	13	23	11	11	15	10	20
S. P. (arheolog)	11	11	24	14	10	16	10	19
M. B. (arheolog)	11	13	23	11	10	15	10	20
M. D. (arheolog)	11	10	23	11	12	14	11	20
R. M. (arheolog)	10	10	23	11	12	14	11	20
T. K. (arheolog)	11	11	23	11	12	14	11	20
M. L. (arheolog)	11	12	23	13	12	15	12	19
P. P. (arheolog)	11	12	23	13	12	14	12	19

Elimination database: DNA typing of mtDNA

Vzorec	Razlike glede na "CRS"	Območje
I. Z. P. (ISM)	HVI: 16343(G) HVII: 73(G), 150(T), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
B. G. P. (ISM)	HVI: 16126(C), 16182(C), 16183(C), 16189(C), 16294(T), 16296(T), 16298(C), 16357(C) HVII: 73(G), 195(C), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
K. V. M. (ISM)	HVI: 16298(C) HVII: 72(C), 263(G), 309.1(C), 309.2(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
B. E. (ISM)	HVI: 16168(T), 16192(T), 16256(T), 16270(T) HVII: 73(G), 150(T), 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
K. P. (ISM)	HVI: 16126(C), 16182(T), 16183(C), 16189(C), 16294(T), 16296(T), 16298(C), 16300(G) HVII: 73(G), 195(C), 200(G), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
K. I. (ISM)	HVI: 16311(C), 16362(C) HVII: 239(C), 263(G), 309.1(C), 309.2(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
R. B. (ISM)	HVI: identična CRS HVII: 152(C), 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
S. P. (arheolog)	HVI: 16298(C) HVII: 195(C), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
M. B. (arheolog)	HVI: 16189(C), 16194(C) HVII: 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
M. D. (arheolog)	HVI: 16362(C) HVII: 239(C), 263(G), 309.1(C), 309.2(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
R. M. (arheolog)	HVI: 16311(C) HVII: 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
T. K. (arheolog)	HVI: identična CRS HVII: 72(G), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
M. L. (arheolog)	HVI: identična CRS HVII: 152(C), 263(G), 315.1(C)	HVI: 16030-16400 HVII: 55-407
P. P. (arheolog)	HVI: 16233(G), 16256(T), 16311(C), 16343(G) HVII: 73(G), 150(T), 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
I. B. Z. (arheolog)	HVI: 16051(G), 16129(C), 16182(C), 16183(C), 16189(C), 16362(C) HVII: 73(G), 152(C), 217(C), 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
D. A. (arheolog)	HVI: 16248(T) HVII: 146(C), 263(G), 309.1(C), 309.2(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
T. L. (arheolog)	HVI: 16192(T), 16259(T), 16270(T), 16311(C) HVII: 73(G), 150(T), 195(C), 263(G), 309.1(C), 315.1(C)	HVI: 16030-16400 HVII: 55-407
T. T. R. (antropolog)	HVI: 16051(G), 16129(C), 16189(C), 16256(T) HVII: 73(G), 152(C), 217(C), 263(G), 315.1(C), 340(T)	HVI: 16030-16400 HVII: 55-407

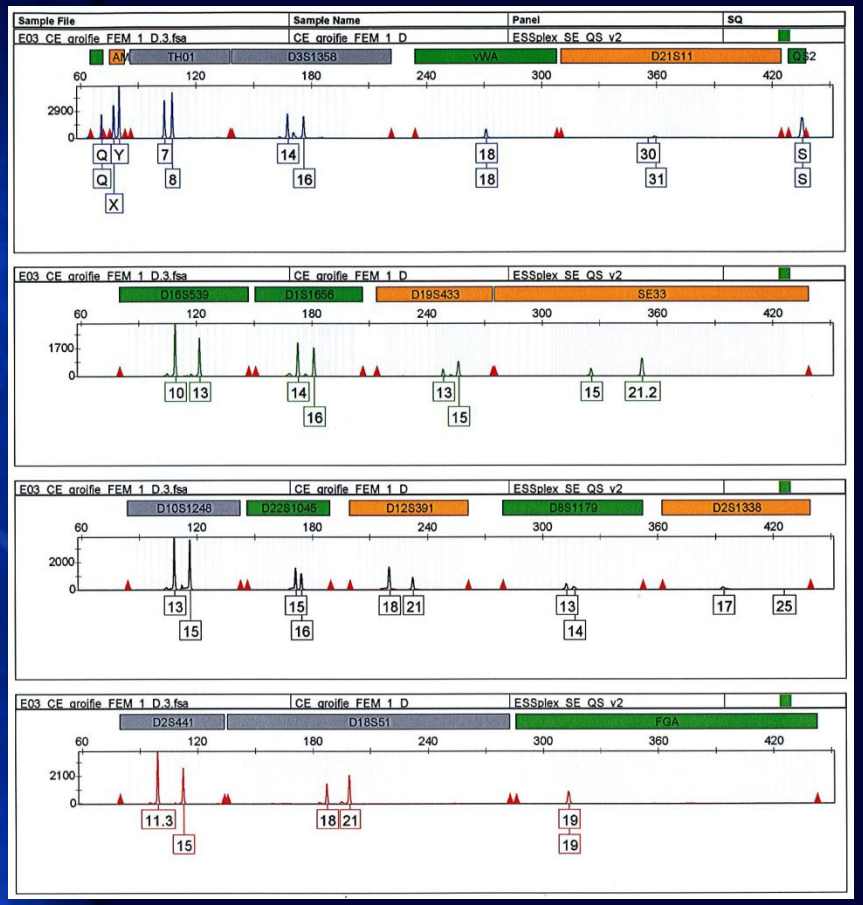
- We are waiting for the family reference samples for comparison with genetic profiles obtained and for identification of the skeleton excavated from Auersperg chapel archaeological site.

Counts of Celje – 15. century

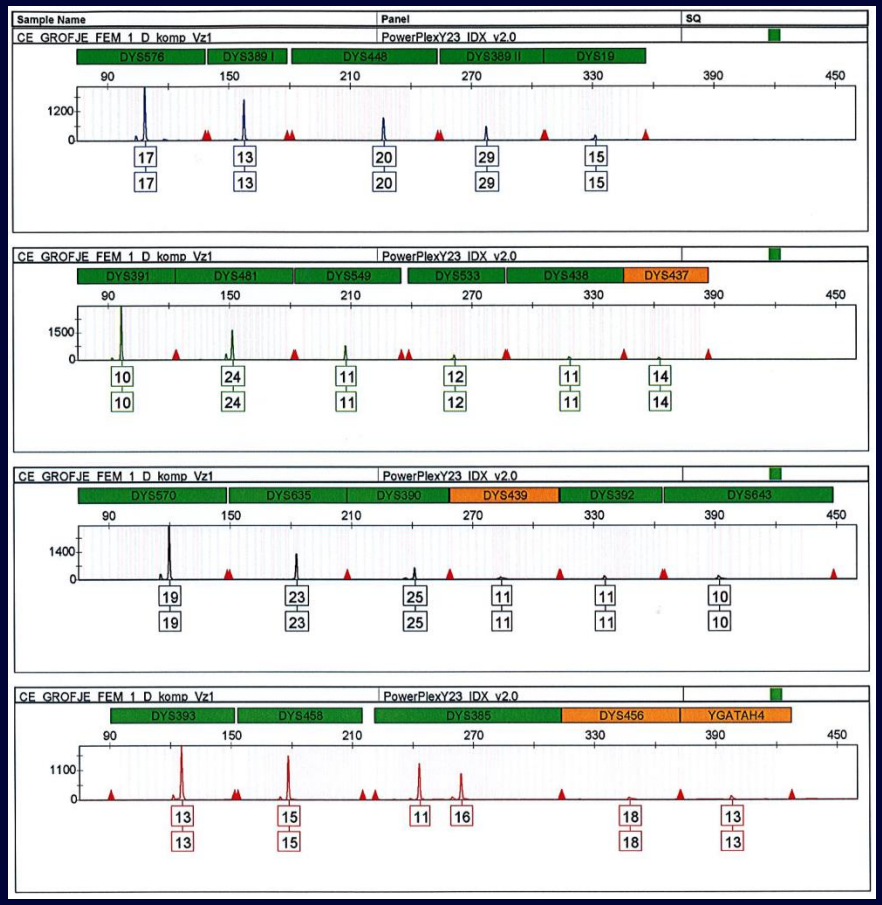
Counts of Celje

Counts of Celje

Counts of Celje

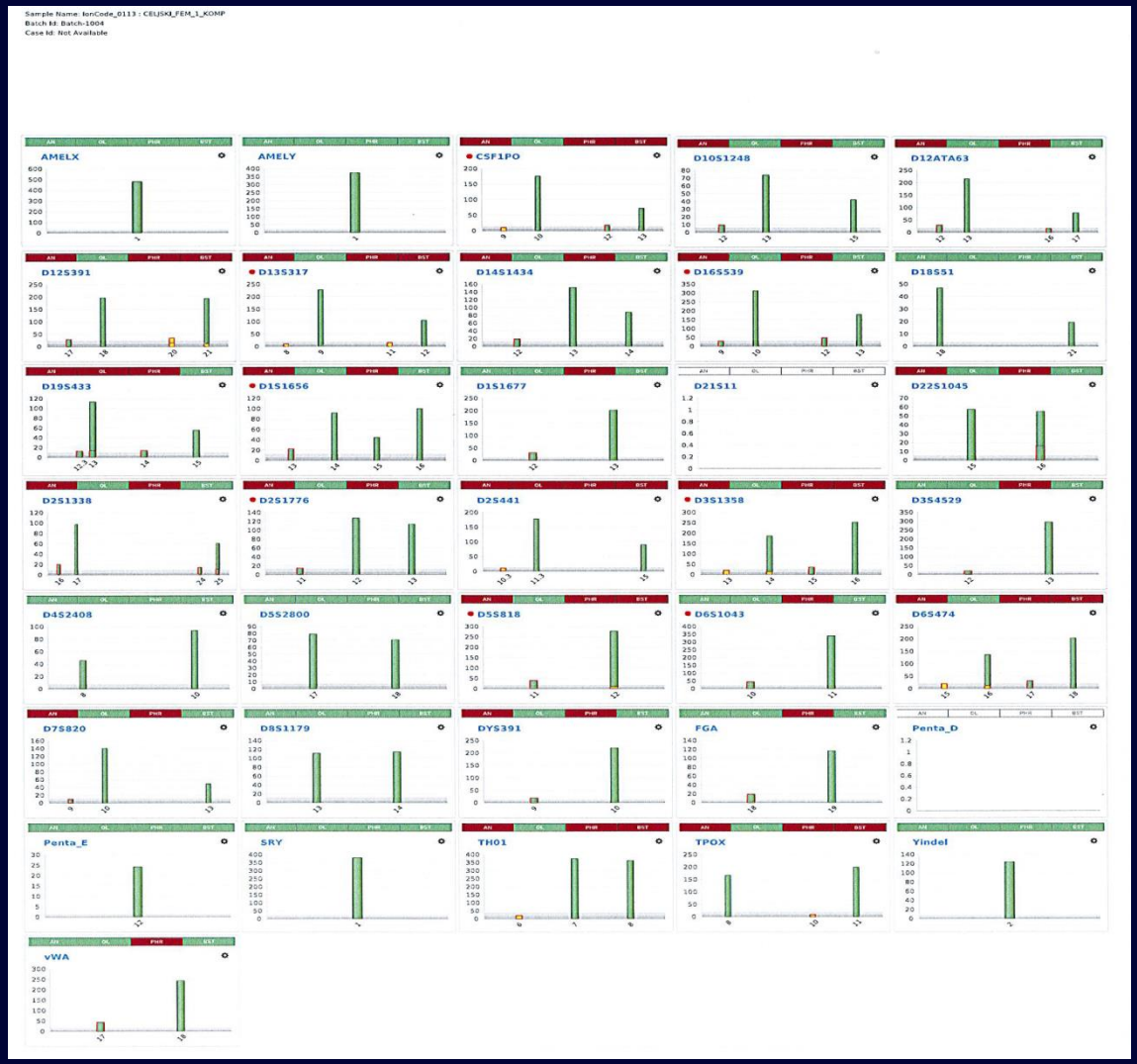


ESSPlex SEQS kit



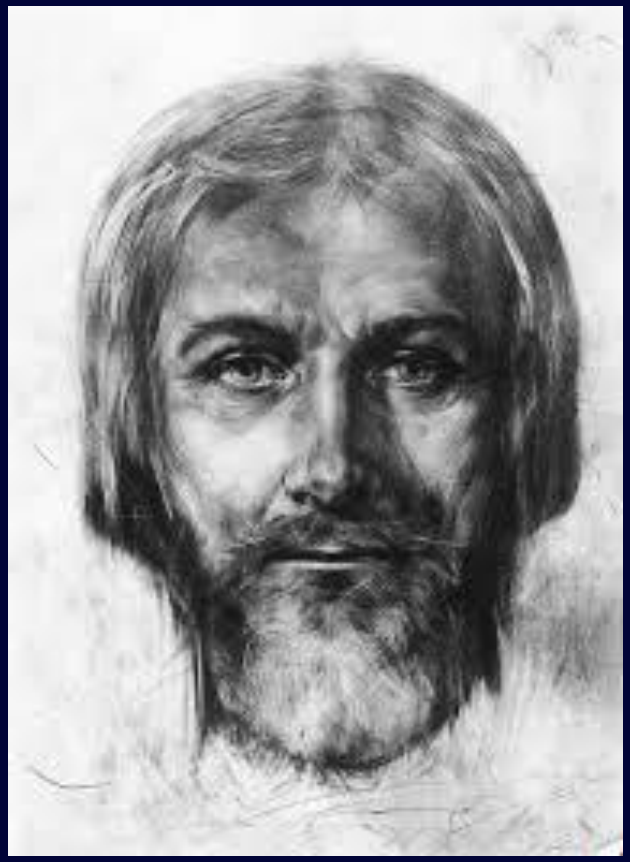
PowerPlex Y23 kit

Counts of Celje



Precision ID GlobalFiler NGS STR Panel (TFS)

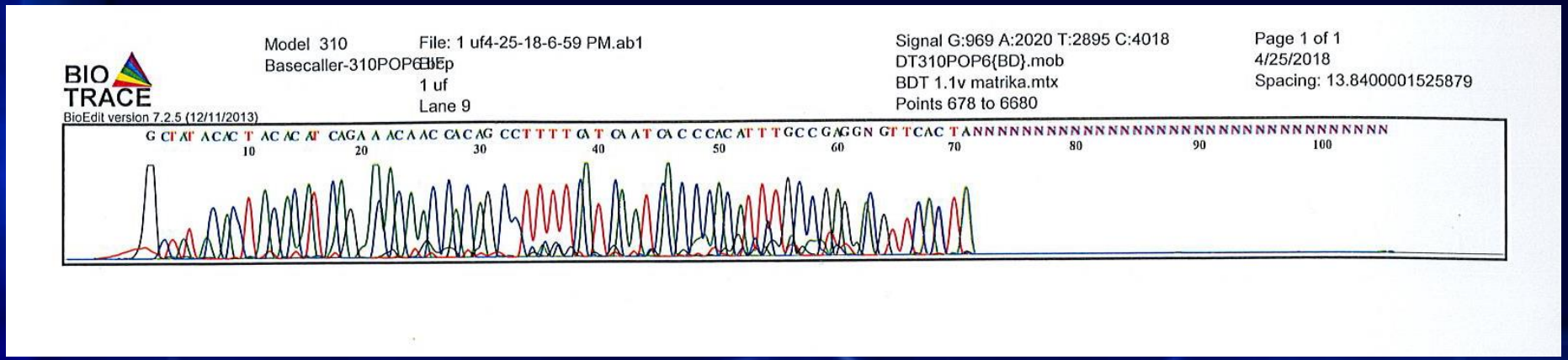
Counts of Celje



Bones in stalagmits from Postojna karst cave (50.000 years old)

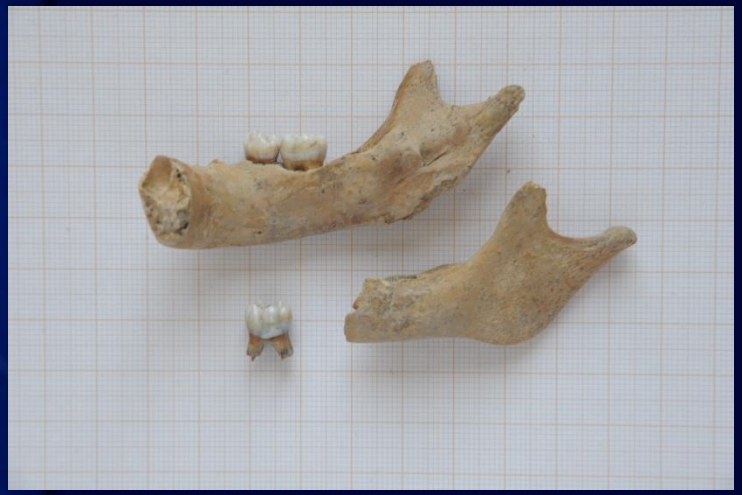
Bones in stalagmits from Postojna karst cave (50.000 years old)

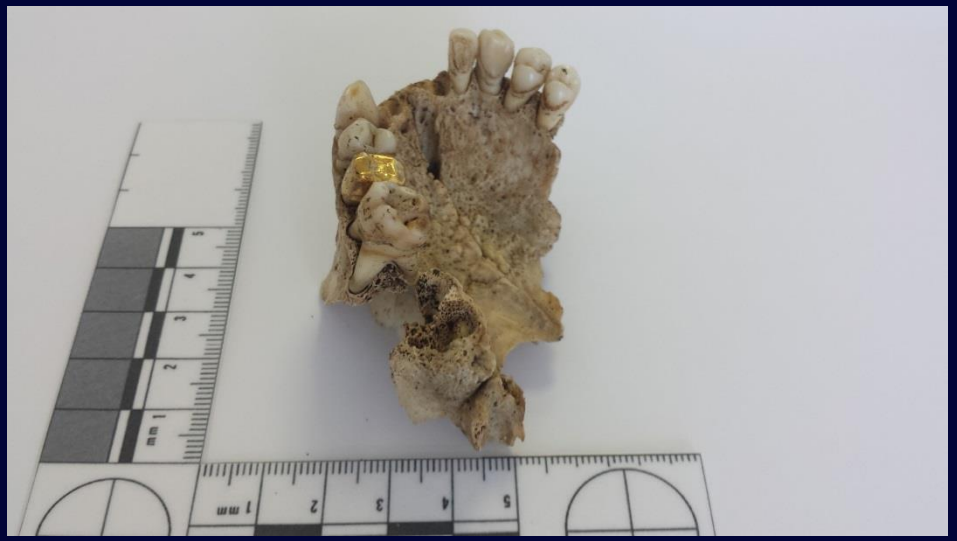
70 bp long sequence of mtDNA Cyt b gene matched the sequence of mtDNA Cyt b gene of cave bear (Ursus spelaeus)



Educational workshops

- **Processing and DNA typing of skeletal remains**





mtDNA Training Course at the Forensic Laboratory in UA Emirates

Sharjah Police Forensic Laboratory

UNITED ARAB EMIRATES
MINISTRY OF INTERIOR
Sharjah Police Headquarters
General Directorate of Police Operations
Forensic Laboratory Section

الإمارات العربية المتحدة
وزارة الداخلية
القيادة العامة لشرطة الشارقة
الإدارة العامة للعمليات الشرطية
قسم المختبر الجنائي

التاريخ: 1435/5/26 هـ
الموافق: 2014/3/27م

Certificate of Appreciation

Sharjah Police wishes to express its gratitude to Dr. Irena Zupanic Pajnic of the University of Ljubljana, Slovenia for her valuable participation to Sharjah Police Forensic Laboratory as an invited speaker and expert consultant in forensic DNA analyses.

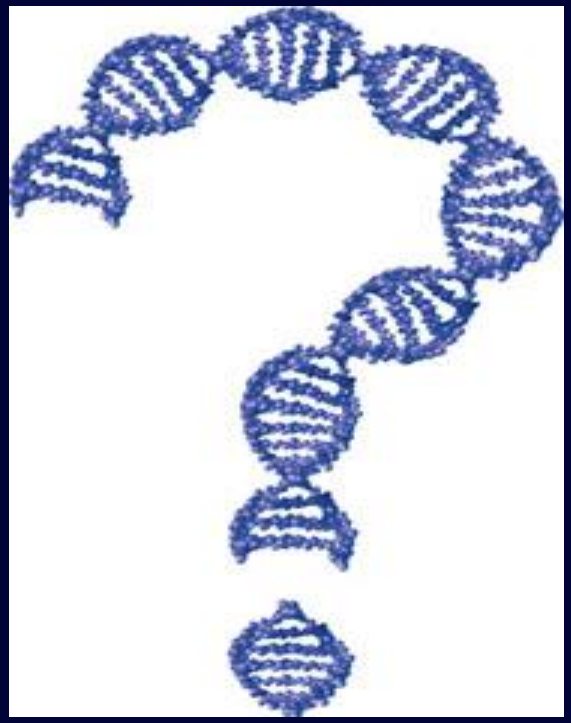
The visit which was held from 23/3/2014 to 27/3/2014 was comprised of, a practical training course covering the implementation of mitochondrial DNA analyses in the Forensic DNA laboratory and a lecture entitled "Genetic identification of Second World War victims in Slovenia".

Sharjah Police in its policy to always improve, is committed to maintain such exchanges and looks forward to further interactions in the future.

Major General
Humaid Mohammed Al Hudaidi
Commander in Chief

P.O.Box: 29 Sharjah,UAE ص.ب 29 الشارقة - الامارات العربية المتحدة Fax: (971 6) 5381122 فاكس: - Tel: (971 6) 5989999 هاتف:
Najeed Service : (Fax :(971 6) 5529000 فاكس ، SMS 7999 رسائل قصيرة : Tel. 800151 هاتف) :خدمة نجيد
Website. www.shjpolice.gov.ae/najeed الموقع الإلكتروني : E-mail : najeed @shjpolice.gov.ae بريد إلكتروني :

THANK YOU FOR YOUR ATTENTION



Feel free to contact me if you have further questions:

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