Forensic genetics

Prof. Univ. Dr Sorin Hostiuc
UMF Carol Davila București

Introduction

Utility of Genetic Analysis in Forensic Medicine

- Identification:
  - Victims in homicides (including infanticide), aviation accidents, fires, nuclear explosions
  - Sexual aggressors - most frequent
  - Perpetrators of robberies, thefts, acts of heteroaggression (including murder)
- Filiation:
  - Paternity testing
  - Maternity testing (accidental child exchanges, abandoned newborns)
  - Identification of illegal adoptions
- Forensic/paleoanthropology/population studies
- Identification of genetic conditions in sudden deaths (e.g., channelopathies)

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Biological Samples That Can Be Used

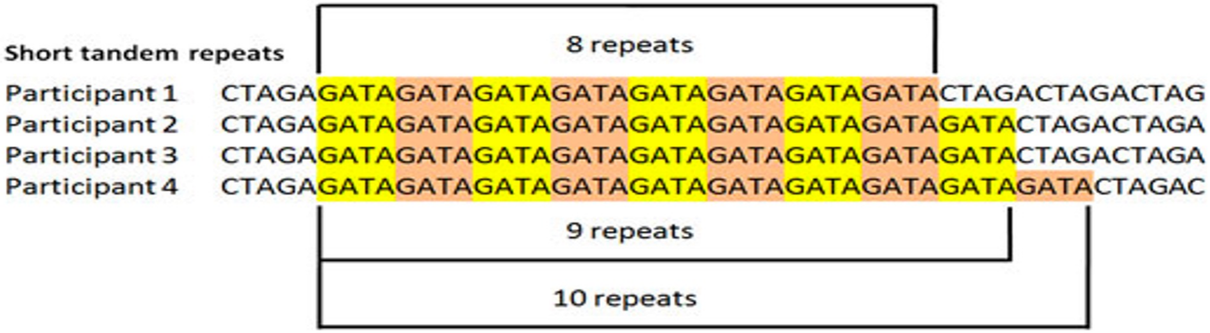
- **Buccal swabs**
- **Blood** - requires somewhat larger quantities, as most cellular elements are anucleate (red blood cells)
- **Skeletal muscle** - collection at the autopsy room is recommended, along with blood
- **Vaginal secretion** - advisable to collect 3 samples in plastic tubes
- **Blood/sperm stains** - minimum recommended diameter - 0.5-1 cm
- **Hair strands** - minimum 10-20 with roots (without roots they are not useful for STR analysis)
- **Other organ fragments** - variable results; if fresh, they pose no problems for obtaining genetic material, but putrefaction and bacterial invasion cause atypical DNA hydrolysis resulting in fragments of variable lengths that lead to result interpretation errors
- **Nails, saliva, urine** - sufficient genetic material can be obtained for analysis
- **Dental pulp** - useful when extreme environmental factors have acted (e.g., very high temperatures) causing massive tissue destruction

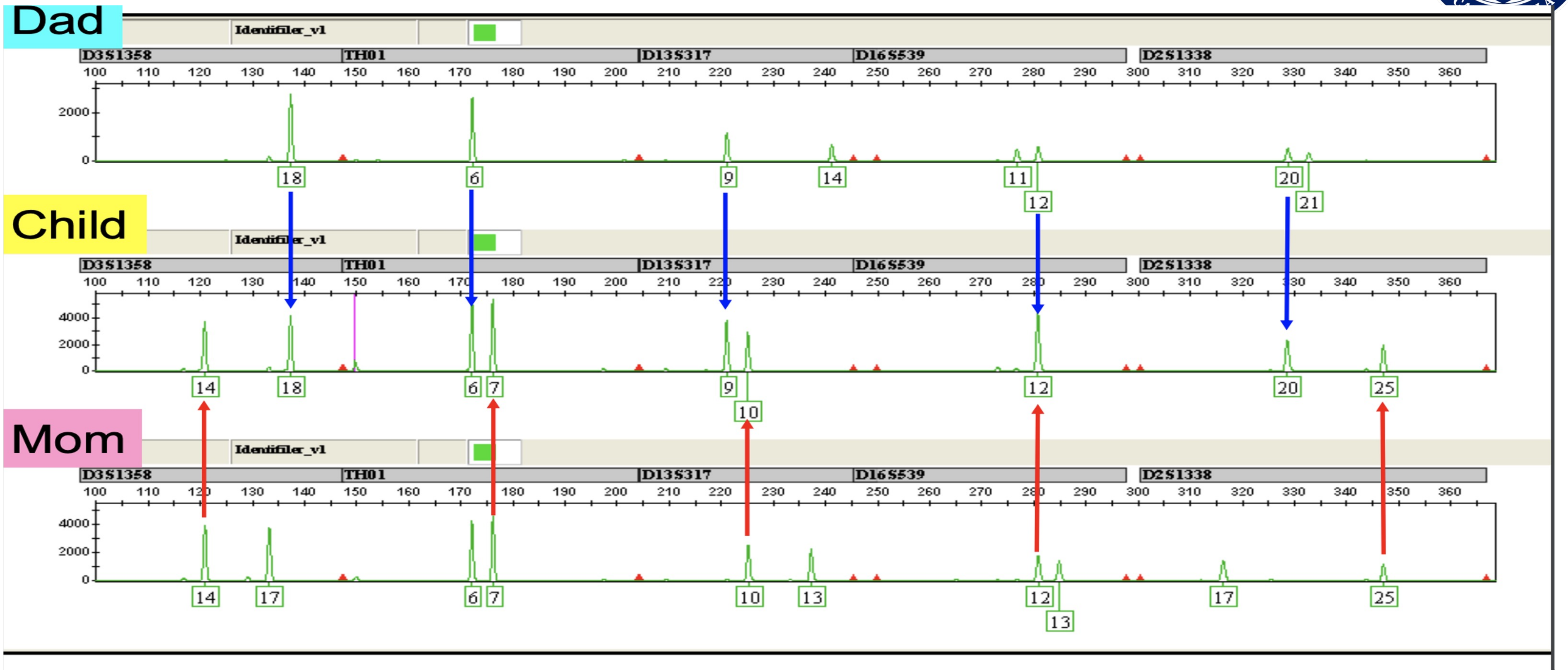
Methods

STR Analysis

- DNA fragments composed of two or more nucleotides (no more than 50) that repeat a variable number of times in a specific DNA region
- Over 10,000 STR sequences described in the human genome
- Most commonly used method in forensic genetics for DNA profiling
- For a specific STR to be used in forensic genetic analyses, it must be:
    - Polymorphic (presents a different number of repetitions in different individuals at the same genetic locus)
    - Unilocus (with unique genome localization)
    - Formed of tetra or penta repetitions
    - With a minimum number of genetic artifacts

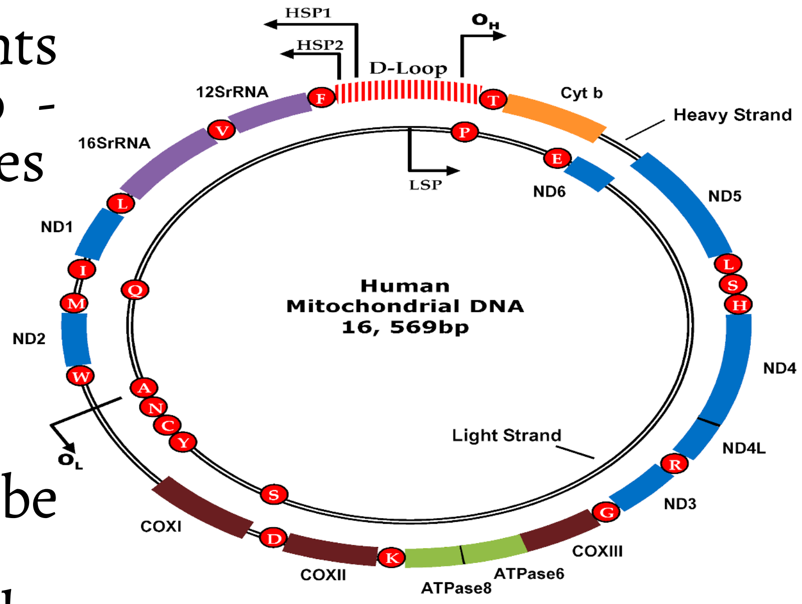
- To identify a person with statistically high probability, analysis of 10-16 STR loci is necessary

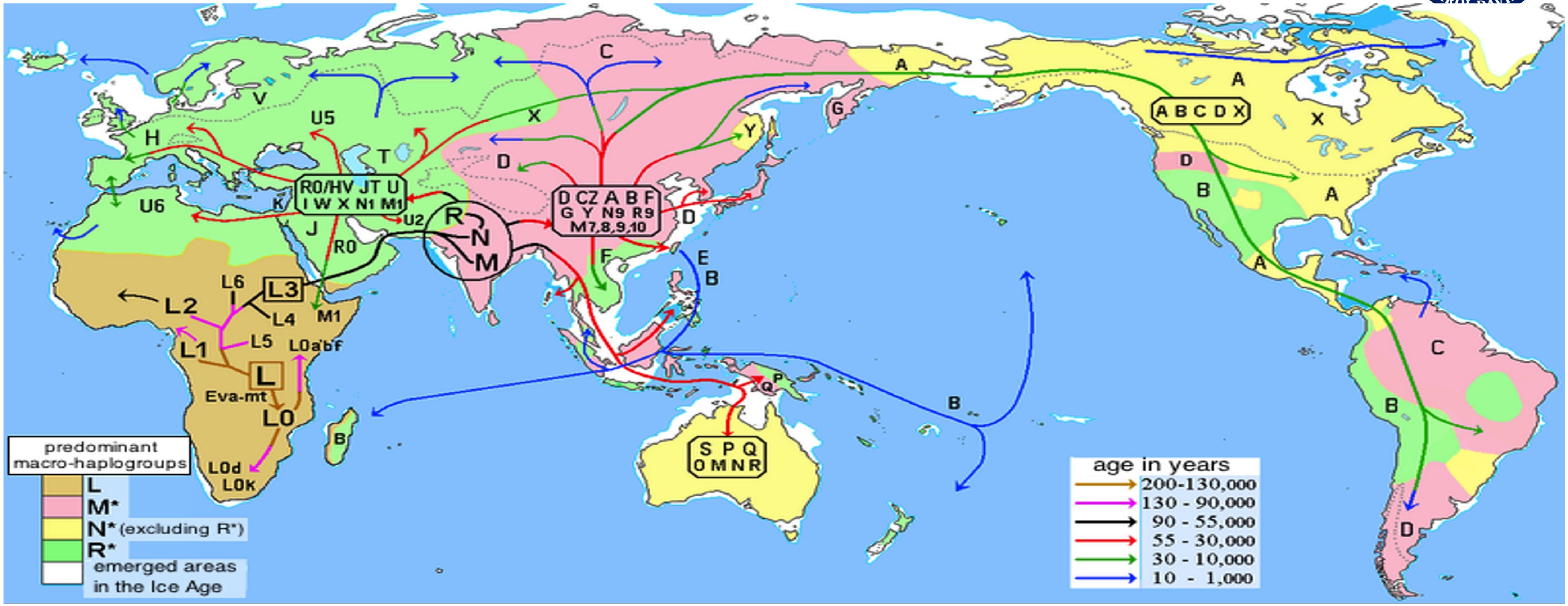



Dad
Identifiler_v1
D3S1358
TH01
D13S317
D16S539
D2S1338
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
2000
0
18
6
9
14
11
12
20
21
Child
Identifiler_v1
D3S1358
TH01
D13S317
D16S539
D2S1338
4000
2000
0
14
18
6
7
9
10
12
20
25
Mom
Identifiler_v1
D3S1358
TH01
D13S317
D16S539
D2S1338
4000
2000
0
14
17
6
7
10
13
12
13
17
25

mtDNA Sequencing

- Maternal line transmission
- mtDNA is circular => greater resistance to degradation
- Does not contain repetitive sequences but presents a highly polymorphic region called the D-loop - analyzed through molecular sequencing techniques
- Maximum utility for:
    - Skeletonized remains
    - Forensic anthropology
    - Filiation (e.g., Romanov case)
- Unlike STR analysis, mtDNA sequencing can be used for single hair strand analysis, without roots
- Disadvantage: not being unique, mtDNA cannot be used for criminal identifications or paternity analysis




predominant macro-haplogroups
L
M*
N* (excluding R*)
R*
emerged areas in the Ice Age
age in years
200-130,000
130 - 90,000
90 - 55,000
55 - 30,000
30 - 10,000
10 - 1,000
L
Eva-mt
L0
L0d
L0k
L0a'b'f
L1
L2
L3
L4
L5
L6
M1
B
R
N
M
R0/HV JT U
I W X N1 M1
U2
K
J
R0
U6
H
V
U5
T
X
C
D
D CZ A B F
G Y N9 R9
M7,8,9,10
Y
G
F
D
E
B
A
S P Q
O M N R
P
Q
B
A B C D X
A
X
D
B
A
A
C
B
D

Y-STR Analysis (STR on Y chromosome)

- High utility in cases where we have a mixture of male and female genetic material
- Markers of choice for paternity testing
- Disadvantage - transmitted through paternal line, so the son cannot be differentiated from the father

SNP Analysis

- Identifies variations of a single nucleotide
- Present in the genome with a frequency of approximately 1/1000 base pairs
- Although their number is much greater than that of STRs, their discriminative capacity is much lower because they are usually biallelic (for each SNP locus there are two variants, while for STRs the number of allelic variations is much greater)
- Using 30-40 different SNP loci has the same discriminative capacity as using 13 STR loci
- Increased utility if working with highly degraded genetic material, as SNPs can be identified on 100-base fragments where STR analysis cannot be performed
- Current use:
    - Forensic anthropology, in mass casualty accidents (World Trade Center victims)
    - Analysis of ethnic/population origin of a person (some SNPs are specific to certain populations)

Teeth as a DNA Source

- Extremely resistant to postmortem phenomena, including putrefaction, autolysis, water immersion, fires up to 1100°C

- Pulpar neurovascular cells and (to a lesser extent) odontoblasts embedded in the predentin layer during dental tubule mineralization are extremely useful for obtaining genetic material when other sources can no longer be used

Methodology

If the tooth **integrity must be preserved** (needed for other anthropometric evaluations or due to cultural/historical importance):

- Horizontal incisions at the enamel-cement junction level, through which the pulp chamber and root pulp system, predentin and dentin are exposed, from where biological samples are taken

E.g., DNA test for the Romanov family, establishing G. Washington's extended family, mass disasters

- Use of a microfluidic pump to wash nucleated cells from dental pulp, followed by capturing them as they pass through micro-orifices at the occlusal surface level (Krzyzanska)

If tooth **integrity** preservation **is not necessary** - crushing and grinding the tooth/teeth into fine powder, maximizing DNA capture

Obtaining DNA from Saliva

- Can be obtained both from the victim's/aggressor's oral cavity and from surfaces
- In rape/sexual assaults - genetic material collection is targeted based on history
  - Control sample collection from the victim's oral cavity is needed
- Before identifying DNA from the saliva sample, must be performed the **identification of the biological product type (is/is not saliva):**
  - **Ultraviolet light.** Advantage - inexpensive, easy to use. Disadvantage - can destroy DNA integrity. NOT recommended
  - **Lasers or high-intensity lights filtered on a single wave**. Advantage - does not destroy DNA integrity. Disadvantage: Less readily available
  - **Biochemical tests** - alpha-amylase identification. Inexpensive (rapid kits), stable in vitro structure. Disadvantage - consumes biological material, leaving less for DNA extractions

Examples

The Romanov Case

- July 1991, 9 corpses were removed from a grave in Ekaterinburg that Russian forensic physicians considered to be those of the last tsar, tsarina, three of their children, the royal physician, and three servants
- To confirm the existence of a family in the grave - STR and mtDNA analysis

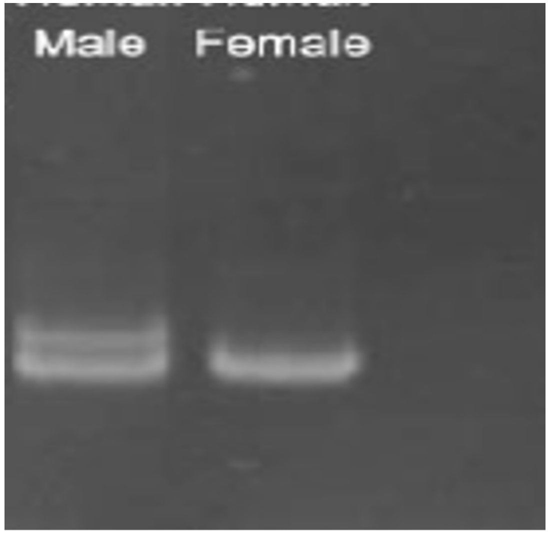
Chromosomal Analysis

Amelogenin test

- Protein involved in dental development
- Gene located on both X (Xp22.1-Xp22.3) and Y (Yp 11.2) chromosomes
- Gene on X chr = AMELX -> leads to appearance of 106bp amplification product (amplicon) (6bp deletion in intron 1 on the gene located on X chromosome)
- Gene on Y chr = AMELY -> leads to appearance of 112bp amplification product (amplicon)

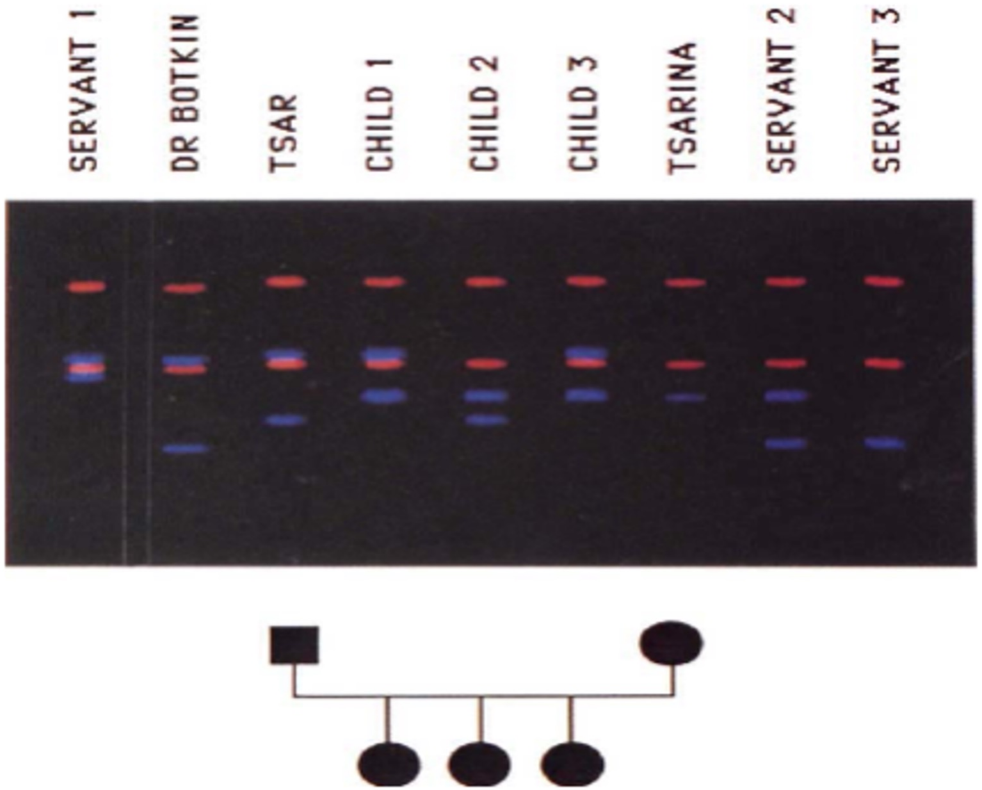
Method:

- Biological product amplification through PCR (specific primers for gene intron 1 are used)
- Amplicon analysis through gel electrophoresis



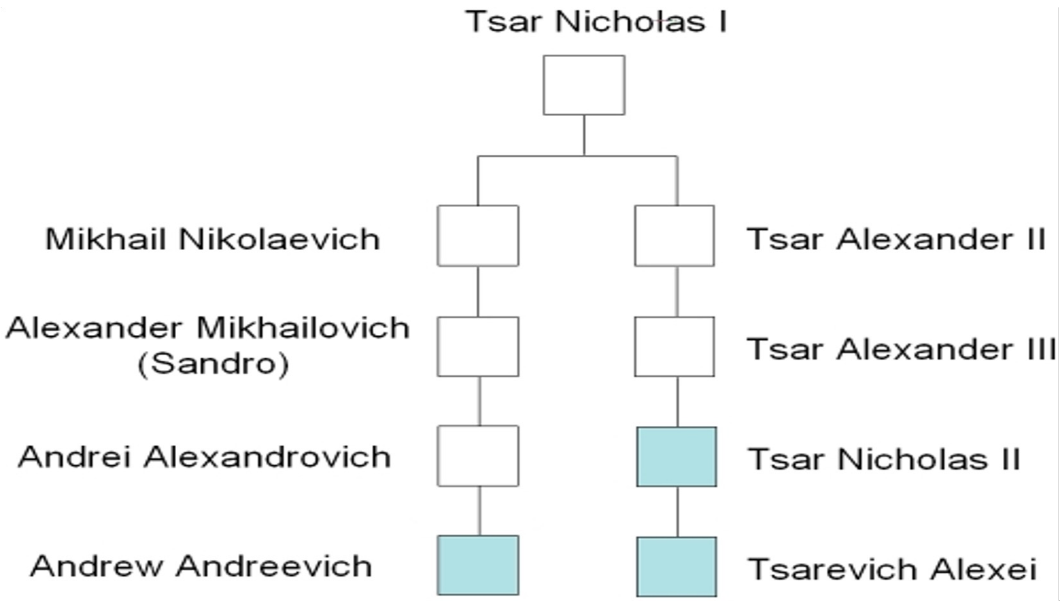
Male - 2 bands in agar gel
Female - one band in agar gel

Analiza STR



- Analiza STR – 5 loci tetramerici

Y-STR



Tsar Nicholas I

Mikhail Nikolaevich

Alexander Mikhailovich (Sandro)

Andrei Alexandrovich

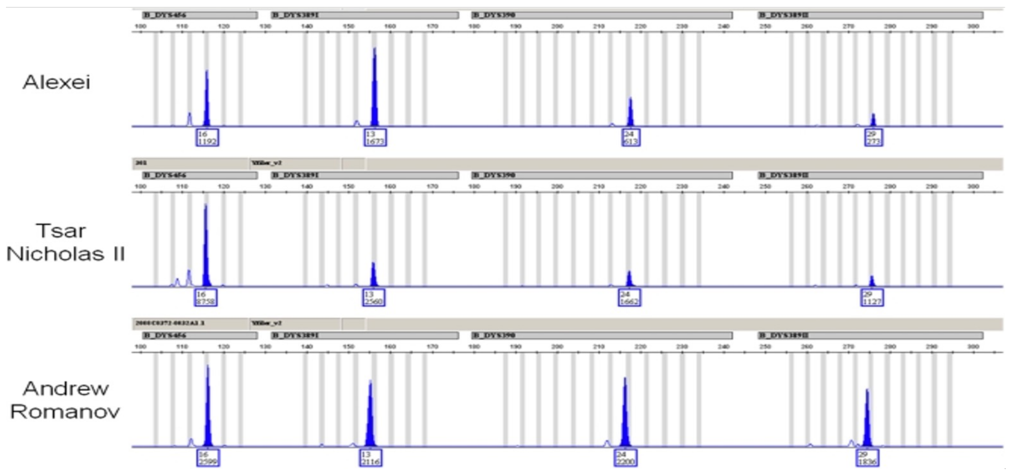
Andrew Andreevich

Tsar Alexander II

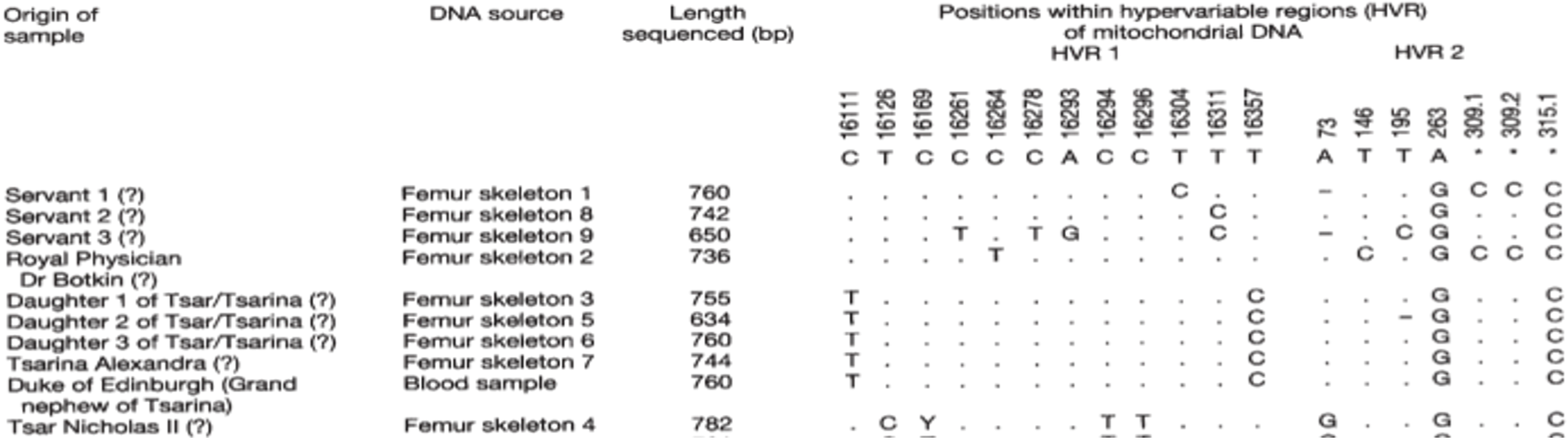
Tsar Alexander III

Tsar Nicholas II

Tsarevich Alexei



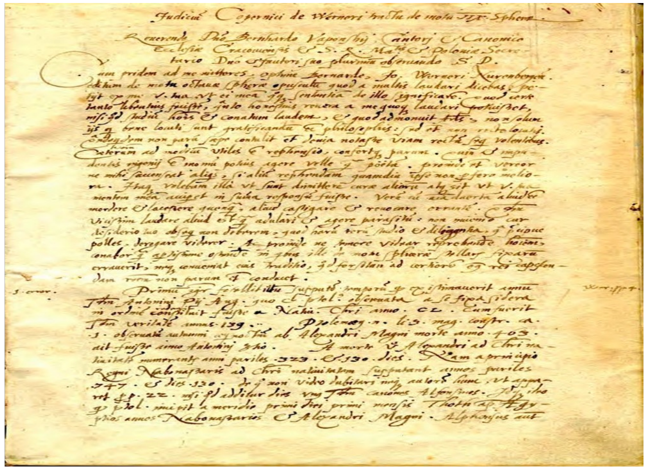
ADNmt



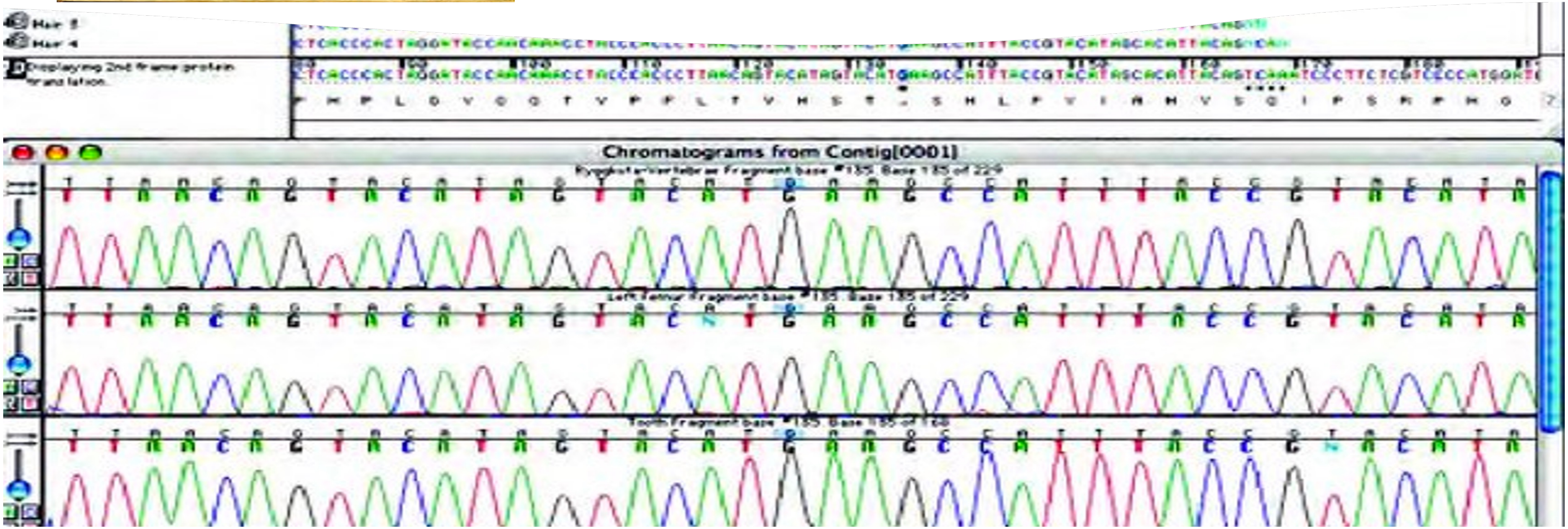
Origin of sample	DNA source	Length sequenced (bp)	Positions within hypervariable regions (HVR) of mitochondrial DNA																		
			HVR 1												HVR 2						
			16111	16126	16169	16261	16264	16278	16293	16294	16296	16304	16311	16357	73	146	195	263	309.1	309.2	315.1
			C	T	C	C	C	C	A	C	C	T	T	T	A	T	T	A	*	*	*
Servant 1 (?)	Femur skeleton 1	760	.	.	.	.	.	.	.	.	.	C	.	.	–	.	.	G	C	C	C
Servant 2 (?)	Femur skeleton 8	742	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	C
Servant 3 (?)	Femur skeleton 9	650	.	.	.	T	.	T	G	.	.	.	C	.	–	.	C	G	.	.	C
Royal Physician Dr Botkin (?)	Femur skeleton 2	736	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	G	C	C	C
Daughter 1 of Tsar/Tsarina (?)	Femur skeleton 3	755	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Daughter 2 of Tsar/Tsarina (?)	Femur skeleton 5	634	T	.	.	.	.	.	.	.	.	.	.	C	.	.	–	G	.	.	C
Daughter 3 of Tsar/Tsarina (?)	Femur skeleton 6	760	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Tsarina Alexandra (?)	Femur skeleton 7	744	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Duke of Edinburgh (Grand nephew of Tsarina)	Blood sample	760	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Tsar Nicholas II (?)	Femur skeleton 4	782	.	C	Y	.	.	.	.	T	T	.	.	.	G	.	.	G	.	.	C

Copernicus

- Was buried in a cathedral in Frombork, on the banks of the Vistula River, but no one knew exactly where
- In 2005, a team of archaeologists explored the Altar of the Holy Cross, one of the cathedral's 16 altars, where they identified 13 skeletons, one of which corresponded to a man of approximately 70 years (possibly Copernicus)



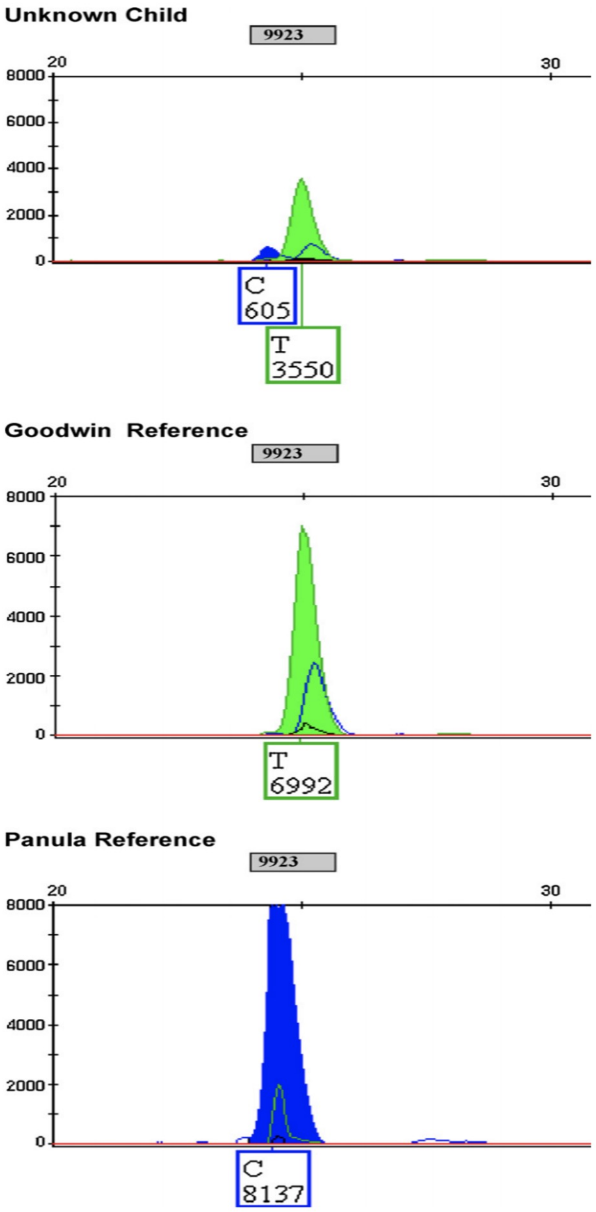
- To be certain of identification, the genetic profile obtained from DNA extracted from teeth was compared with mtDNA from hair strands found in the book Calendarium Romanum Magnum, written by Johannes Stoeffler, which Copernicus owned his entire life. The genetic profiles were identical, thus achieving positive identification



The child from the Titanic

- April 15, 1912, the Titanic sank, their bodies never being found
- April 21, the body of a child was discovered, who was buried in a cemetery in Halifax, Canada
- 1998, the body was exhumed at the request of the Palsson family from Sweden, who believed the child was Gosta Leonard Pwallson, who was 2 years and three months old at the time of the Titanic's sinking

- During exhumation, three teeth were recovered (55, 73, 84)
- Of the three teeth, 84 contained dentin while 55 and 73 did not
- Based on these results, it was established that the child's age was 9-15 months, unable to be 27 months old



- Subsequently, researchers from Brigham Young University in Utah isolated mtDNA from dentin and compared the profile with descendants of all mothers who were on the Titanic and had children under 3 years who died in the accident
- The obtained profile suggested the possibility that he was either a 19-month-old boy or a 13-month-old one
- Considering that the first brother was too old, it was concluded that the body belonged to a 13-month-old child named Eino Viljam Panul

- Subsequently, however, a pair of shoes was identified that belonged to the child, which the police officer who discovered the body had kept
- These shoes were too large to be worn by a 13-month-old child
- A new genetic analysis (mtDNA) was performed, which identified a difference at the level of a base pair, the profile being identical to that of the 19-month-old girl
- Therefore, based on the new data, it was concluded that the skeleton belonged to a girl named Sidney Goodwin