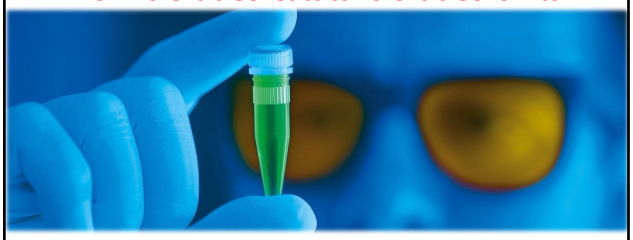
MCBD II Core Lecture - Mar 14 2024

From Forensic Genetics to Forensic Genomics



Dr. Walther Parson
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walther.parson@i-med.ac.at

1

CSI laboratory (ISO17025)
Austrian Central DNA laboratory
EDNAP, ENFSI, Interpol
Int. reference lab for forensic DNA testing

DVI laboratory (ISO17025)
e.g. Tsunami (Sri Lanka, 2004), Chile (1973 regime victims), Missing Mexican students (2014)

Forensic molecular research laboratory
Mitochondrial DNA databasing (EMPOP)
Population genetics (mito, Y)
Forensic DNA Phenotyping (appearance, ancestry, age)
New technologies (Massively Parallel Sequencing)

4

Locard's exchange principle

A perpetrator of a crime
will bring something into the crime scene
and leave with something from it

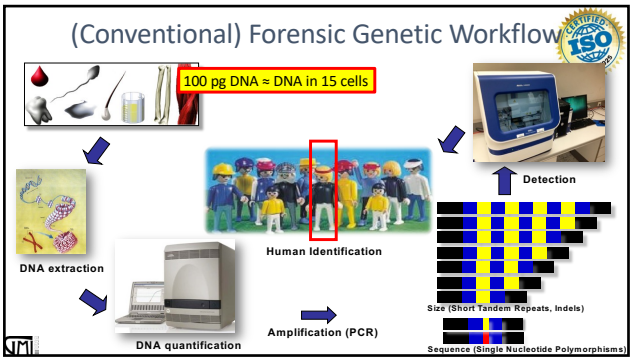
Both can be used as **forensic evidence**

A C G T

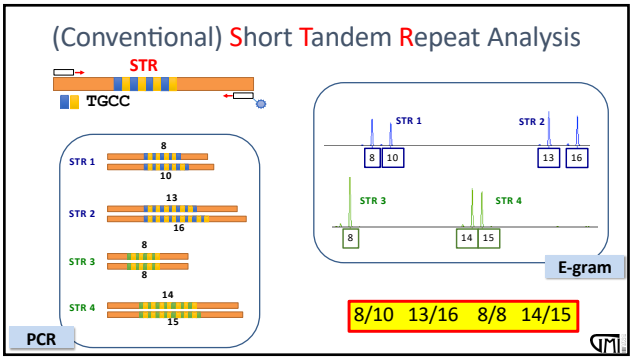
Edmond Locard
(1877-1966)

W. de Gruyter, 1916

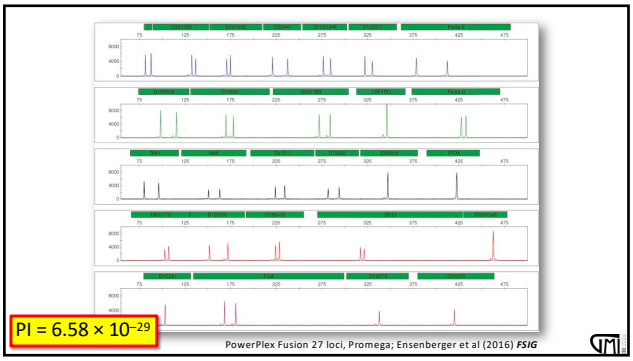
5


(Conventional) Forensic Genetic Workflow
100 pg DNA ≈ DNA in 15 cells
DNA extraction
Human Identification
DNA quantification
Amplification (PCR)
Detection
Size (Short Tandem Repeats, Indels)
Sequence (Single Nucleotide Polymorphisms)

6


(Conventional) Short Tandem Repeat Analysis
STR
TGCC
STR 1
STR 2
STR 3
STR 4
PCR
E-gram
8/10 13/16 8/8 14/15

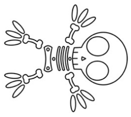
7


PI = 6.58 × 10−29
PowerPlex Fusion 27 loci, Promega; Ensenberger et al (2016) FSIG

8

Applications

Crime casework
reference sample



Human identification
ante mortem sample
or
related person(s)

Paternity casework
related person(s)

Cell line ID

9

European Network of Forensic Science Institutes

DNA Intelligence Databases

Welcome to the ENFSI Portal

Country	Population size	Persons A	Persons S	Persons CO	Persons T	Stains	Matches Person/Stain S	Matches Person/Stain CO	Matches Person/Stain T	Matches Stain/Stain	Matches Total	Date	Stain-person matches per person
Austria	8,100,000				134,938	39,305			12,404	5,411	17,815	Jul-10	0.09
Belgium	10,400,000		n/a	19,591	19,591	21237	258	1,081	1,339	2,359	3,698	Jun-10	0.07
Bulgaria	7,900,000				17,618	1,147			377	122	499	Jul-09	0.02
Croatia	4,600,000				29,293	5,180			3,752	1,519	5,271	Feb-10	0.13
Cyprus	772,000												
Czech Republic	10,300,000												
Denmark	5,500,000				61,397	35,796			11,774	3,411	15,185	Jun-10	0.19
Estonia	1,400,000				29274	9376			2860	895	3,755	jul. 09	0.10
Finland	5,360,000				97,113	11,534			12161	1,503	13,664	Jun-10	0.13
France	59,300,000	113,537	1,075,308	314,080	1,389,388	79,670	24,727	8,499	33,226	5,352	38,578	June-10	0.02
Georgia	4,700,000												
Germany	81,835,000				692,284	176,480			79,701	22,282	101,983	Jun-10	0.12
Greece	10,600,000												
Hungary	10,200,000				81,812	2,065			127	149	276	Jun-10	0.00
Ireland	4,200,000												
Italy	58,000,000												
Latvia	2,400,000												
Lithuania	3,369,000				44,877	4,260					1,017	Jun-10	0.00
Luxembourg	500,000		53	756	809	515	27	60	87	464	551	Jul-10	0.11
Former Yugoslavian Republic of Macedonia	2,000,000				1,935	1,812					356	Apr-10	0.00
Malta	400,000												
Netherlands	16,100,000		14,721	85,867	100,588	43,006			23,256	5,002	28,258	Jun-10	0.23
Northern Ireland	1,685,000												
Norway	4,500,000		5,809	16,283	22,092	6,162			2,066	705	2,771	Jun-10	0.09
Poland	38,200,000				25,599	1,807			101	68	169	40359	0.00
Portugal	10,300,000												
Romania	22,000,000			5,820	5,820	70		1	1		1	Sep-09	0.00
Russia	143,800,000												
Scotland	5,062,000		130,809	94,725	236,202	9,987			18,410	2,046	20,456	Jul-08	0.08
Slovakia	5,500,000				21,731	5,226			1,638	750	2,388	Jun-10	0.08
Slovenia	2,000,000				13,541	3,750			1,934	311	2,245	Dec-09	0.14
Spain	44,800,000				110,357	47,073			12,357	19,831	32,188	aug-10	0.11
Sweden	9,000,000		22160	62239	84,399	20,839	20,249	5,992	26,241	14,194	40,435	Jul-10	0.31
Switzerland	7,779,000				118,256	27,918			24,965	6,054	31,019	Jun-10	0.21
Turkey	66,800,000												
UK (England & Wales)	53,700,000				5,042,20	365,78			1,046,128	87,930	1,134,058	Jun-10	0.21
Ukraine	47,600,000												
Total	**770,662,000**				**8,381,115**	**919,997**			**1,314,905**	**180,358**	**1,496,636**		

A = Arrestees
S = Suspects
CO = Convicted offenders
T = Totals (or when no distinction can be made)

http://www.enfsi.org/

COLD HIT RATE > 25%

10

HID of Historic Individuals

Leopold III (+1136) — Bauer et al. 2013
King Richard III (+1485) — King et al 2014
Wolfgang A. Mozart (+1791) — Parson 2006
Friedrich v. Schiller (+1805) — Parson 2008
Romanov family (+1918) — Coble et al 2009

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The Identification of the Romanov Family

Tsar family (1913) 300th Anniversary of the Romanov Dynasty

12

The Royal family was held under house arrest by the Bolsheviks in the Ipatiev House, Ekaterinburg (April - July 1918)

Nikolay Sokolov (1919)
Judge and Chief Investigator

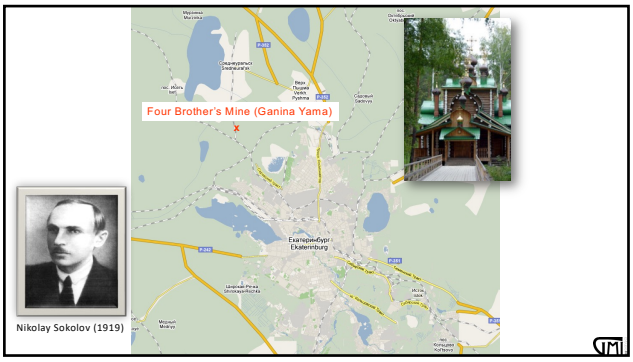
From the Sokolov collection (Harvard)

13

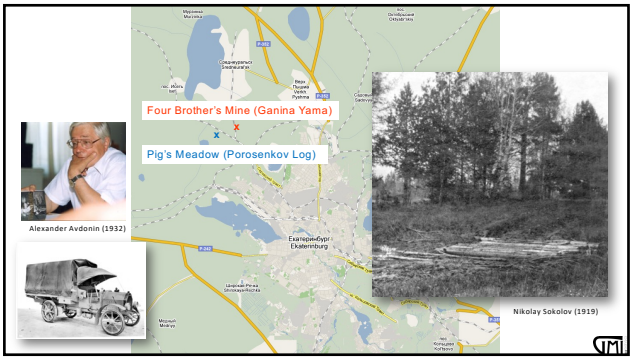
Basement room of the Ipatiev House where the Russian Imperial family was murdered July 16-17 1918 by members by Bolsheviks

From the Sokolov collection (Harvard)

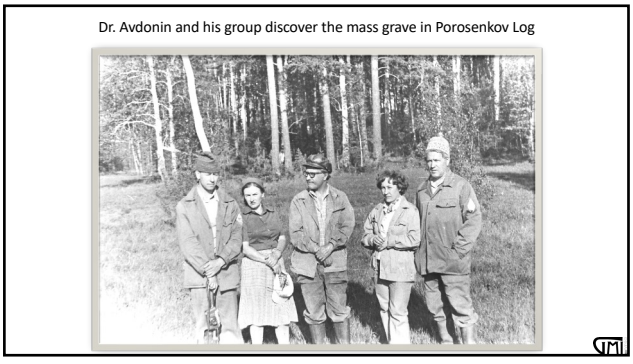
14


Four Brother's Mine (Ganina Yama)
Nikolay Sokolov (1919)

15


Four Brother's Mine (Ganina Yama)
Pig's Meadow (Porosenkov Log)
Alexander Avdonin (1932)
Nikolay Sokolov (1919)

16

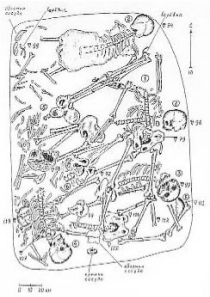

Dr. Avdonin and his group discover the mass grave in Porosenkov Log

17

Dr. Avdonin and his group planting bushes to hide their find

18

1991 – Official discovery of the mass grave and excavation of the remains



19

Identification of the remains of the Romanov family by DNA analysis

Peter Gill[1], Pavel L. Ivanov[2], Colin Kimpton[1], Romelle Piercy[1], Nicola Benson[1], Gillian Tully[1], Ian Evett[1], Erika Hagelberg[3] & Kevin Sullivan[1]

Nature Genetics – Feb. 1994

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Anna Demidova
Alexei Trupp
Ivan Kharitinov
Dr. Eugene Botkin

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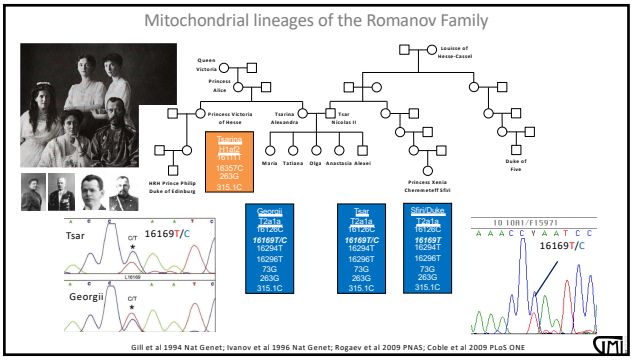
Mitochondrial lineages of the Romanov Family
Queen Victoria
Princess Alice
Princess Victoria of Hesse
HRH Prince Philip Duke of Edinburg
Tsarina Alexandra
Tsar Nicolas II
Maria
Tatiana
Olga
Anastasia
Alexei
Louise of Hesse-Cassel
Princess Xenia Chevmotieff Sisi
Duke of Fife
Tsar
16169T/C
Georgii
16169T/C
Gill et al 1994 Nat Genet; Ivanov et al 1996 Nat Genet; Rogaev et al 2009 PNAS; Coble et al 2009 PLoS ONE

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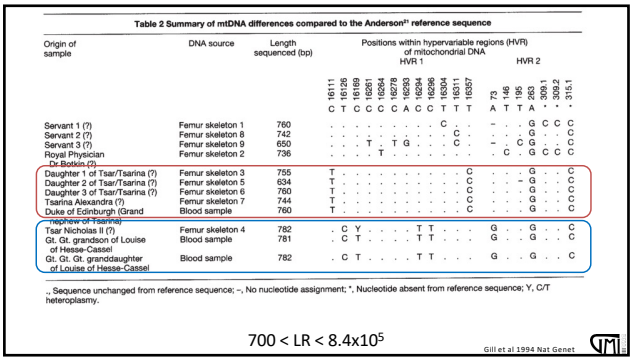
Table 2 Summary of mtDNA differences compared to the Anderson[23] reference sequence
Origin of sample
DNA source
Length sequenced (bp)
Positions within hypervariable regions (HVR) of mitochondrial DNA
HVR 1
HVR 2
Servant 1 (?)
Servant 2 (?)
Servant 3 (?)
Royal Physician Dr Botkin (?)
Daughter 1 of Tsar/Tsarina (?)
Daughter 2 of Tsar/Tsarina (?)
Daughter 3 of Tsar/Tsarina (?)
Tsarina Alexandra (?)
Duke of Edinburgh (Grand nephew of Tsarina)
Tsar Nicholas II (?)
Gt. Gt. grandson of Louise of Hesse-Cassel
Gt. Gt. granddaughter of Louise of Hesse-Cassel
Femur skeleton 1
Femur skeleton 8
Femur skeleton 9
Femur skeleton 2
Femur skeleton 3
Femur skeleton 5
Femur skeleton 6
Femur skeleton 7
Blood sample
Femur skeleton 4
Blood sample
Blood sample
., Sequence unchanged from reference sequence; –, No nucleotide assignment; *, Nucleotide absent from reference sequence; Y, C/T heteroplasmy.
700 < LR < 8.4x10^5
Gill et al 1994 Nat Genet

23

Prince Alexei, aged 8 or 9, in a detail from an official Russian royal-family photograph taken in 1913. His sister Grand Duchess Anastasia's hand drapes over his shoulder.

Remains of Czar Nicholas II's Son May Have Been Found

- FRIDAY, **AUGUST 24, 2007**
- **MOSCOW — The remains of the last czar's hemophiliac son and heir to the Russian throne, missing since the royal family was gunned down nine decades ago by Bolsheviks in a basement room, may have been found, an archaeologist said Thursday.**

http://www.foxnews.com/story/0,2933,294360,00.html

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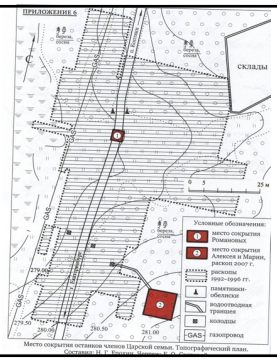

Место сокрытия останков членов Царской семьи. Топографический план.

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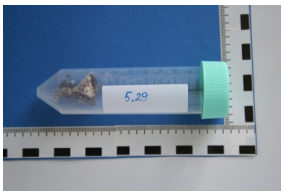
DNA Analyses Innsbruck



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Bone preparation

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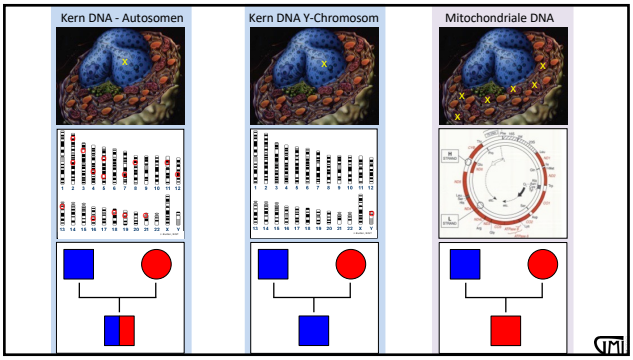

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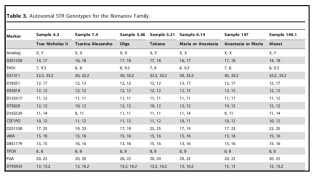
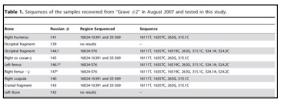
The Identification of the Romanov Family

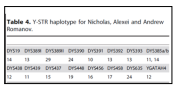
autosomal STRs



Y-STRs

mtDNA





Coble et al ***PLoS ONE*** (2008)

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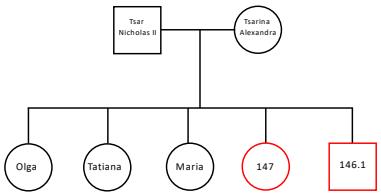
The Identification of the Romanov Family



Hypothesis: Samples 146.1 and 147 are members of the Romanov Family vs. Samples 146.1 and 147 are unrelated

Cumulative LR = **4.36 Trillion**

Coble et al ***PLoS ONE*** (2008)

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Confirmation of the Tsar's STR profile

Nagasaki, 1891

blood stain on shirt

Full matches in Y-Chromosomal and autosomal STR profiles

Coble et al ***PLoS ONE*** (2008)

31

The Imposters

Establishing the identity of Anna Anderson Manahan

Peter Gill, Colin Kimpton, Rosemary Aliston-Greiner, Kevin Sullivan, Mark Stoneking, Terry Melton, Julian Nott, Suzanne Barrit, Rhonda Roby, Mitchell Holland & LTC Victor Weedn

Nature Genetics 9, 9–10 (1994)

Anna Anderson Manahan

Table 2 Mitochondrial DNA sequences

Origin of sample	DNA source	Length sequenced (bp)	Position within the non-coding region (ref. 11)					
			16111	16126	16266	16294	16304	16357
Reference sequence	–	–	C	T	C	C	T	T
Duke of Edinburgh (Great nephew of Tsarina)	Blood sample	403	T	.	.	.	.	C
Anna Anderson	Intestine sample	403	.	C	T	T	C	.
Anna Anderson	Hair sample	344–362 (3 hairs)	.	C	T	T	C	.
C. Maucher (Great nephew of Schanzkowska	Blood sample	380	.	C	T	T	C	.

., Sequence unchanged from reference sequence.

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"They never answered the other forensic evidence — the mountain of forensic evidence— in her favor," says a still-believing Robert Crouch, shown last month placing flowers near the memorial marker of the late Anna Manahan.

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The Imposters

Total number of "Russian imperial children" claimants since 1918:

Olga: 28 claimants

Tatiana: 33 claimants

Maria: 53 claimants

Anastasia: 33 claimants

Alexei: 81 claimants

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PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Mystery Solved: The Identification of the Two Missing Romanov Children Using DNA Analysis

Michael D. Coble, Odile M. Loreille, Mark J. Wadhams, Suni M. Edson, Kerry Maynard, Carna E. Meyer, Harald Niederstätter, Cordula Berger, Burkhard Berger, Anthony B. Falsetti, Peter Gill, Walther Parson, Louis N. Finelli

Published: March 11, 2009 • https://doi.org/10.1371/journal.pone.0004838

241 Save | 120 Citation | 203,949 View | 114 Share

Article | Authors | Metrics | Comments | Media Coverage

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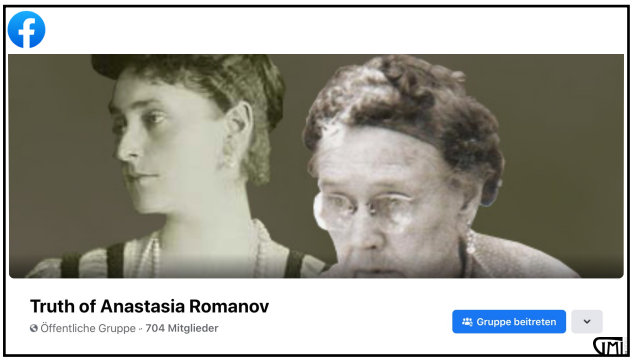

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Irren ist menschlich

Bestätigungsfehler (confirmation bias):

Menschen versuchen Informationen zu finden, die ihre Auffassung **bestätigen**. Im Gehirn werden – ohne dass es bewusst wird – Informationen hochgradig selektiv verarbeitet, sodass individuelle Überzeugungen beibehalten werden. **Suche** nach Informationen, die gegen die eigentliche Überzeugung sprechen.

Dunning-Kruger-Effekt:

Menschen neigen dazu, ihr eigenes Wissen und Können regelmäßig zu **überschätzen**. Wenn wir von einem Themengebiet keine Ahnung haben, ist uns oft nicht bewusst, wie unwissend wir wirklich sind. Die Selbstüberschätzung ist sogar besonders groß, je weniger wir wissen (auch Journalisten, Wissenschaftler,...).

"Gesunder Menschenverstand" (gut feeling error):

Jede Behauptung muss mit Beweisen belegt und eine Argumentation widerspruchsfrei sein. Man wird leicht **manipulierbar**, wenn vernünftiges Denken hinter dem emotionalen Effekt einer Behauptung zurücktritt. Bauchentscheidungen sind anfällig für eine Vielzahl irrationaler Denkmechanismen. Insbesondere **Angst** ist ein starkes Gefühl, von dem sich Menschen sehr stark leiten lassen.

mod von Markus Knauff (Kognitivforscher)

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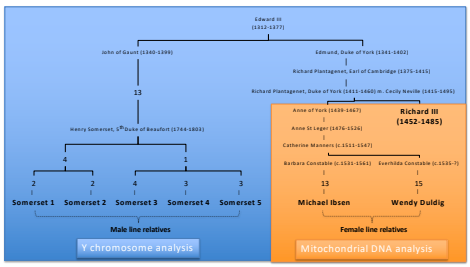
Received 5 Aug 2014 | Accepted 21 Oct 2014 | Published 2 Dec 2014 DOI: 10.1038/ncomms6631 OPEN

Identification of the remains of King Richard III



King et al *Nature Comm* (2014)

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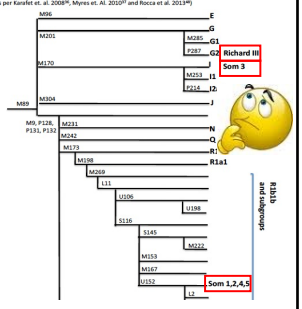
Supplementary Table 6: Mitochondrial DNA type of ML1, ML2 and Skeleton 1.

Position	Reference	Maternal Lineage 1	Maternal Lineage 2	Grey Friars Skeleton 1
73	A	G	G	G
146	T	C	C	C
185	G	A	A	A
188	A	G	G	G
263	A	G	G	G
295	C	T	T	T
310	TCCCCC	TCCCCCC	TCCCCCC	TCCCCCC
462	C	T	T	T
489	T	C	C	C
750	A	G	G	G
1438	A	G	G	G
2706	A	G	G	G
3010	G	A	A	A
4216	T	C	C	C
4769	A	G	G	G
7028	C	T	T	T
8860	A	G	G	G
8994	G	G	A	G
10398	A	G	G	G
10685	G	A	A	A
11251	A	G	G	G
11719	G	A	A	A
12397	A	G	G	G
12612	A	G	G	G
13281	T	C	C	C
13708	G	A	A	A
13933	A	G	G	G
14766	C	T	T	T
14798	T	C	C	C
15326	A	G	G	G
15452	C	A	A	A
16069	C	T	T	T
16126	T	C	C	C
16519	T	C	C	C

Y chromsome haplogroup information



King et al *Nature Comm* (2014)

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Evidence	LR	Posterior – sceptical prior	Posterior – 50/50 prior
All	**6.7 million**	**0.999994**	**0.9999999**
Genetic	79	0.67	0.987
Non-genetic	85,000	0.9995	0.999988
All exc. mtDNA	14,000	0.997	0.99993
All exc. Y	41 million	0.999999	1.0000000
mtDNA only	478	0.92	0.998
For illustrative purposes, below we give likelihood ratios calculated using the European mitochondrial DNA control region database			
mtDNA only	6847	0.994	0.9999
Genetic	1127	0.97	0.999
All	96 million	0.9999996	0.99999999

King et al *Nature Comm* (2014)

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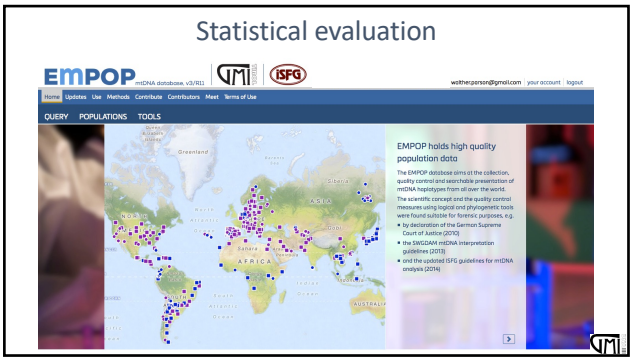
Statistical evaluation
EMPOP
QUERY
POPULATIONS
TOOLS
EMPOP holds high quality population data

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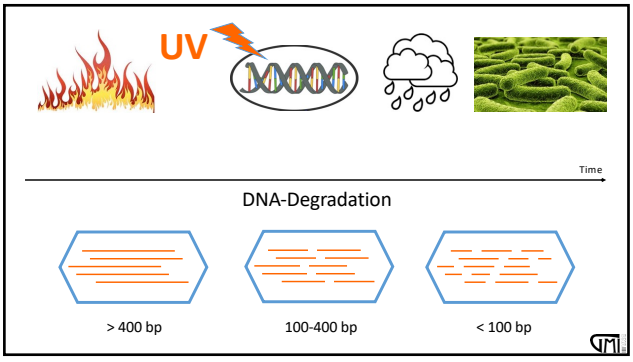
UV
Time
DNA-Degradation
> 400 bp
100-400 bp
< 100 bp

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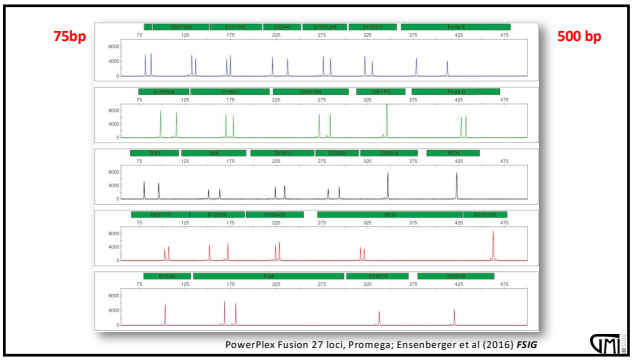
75bp
500 bp
PowerPlex Fusion 27 loci, Promega; Ensenberger et al (2016) FSIG

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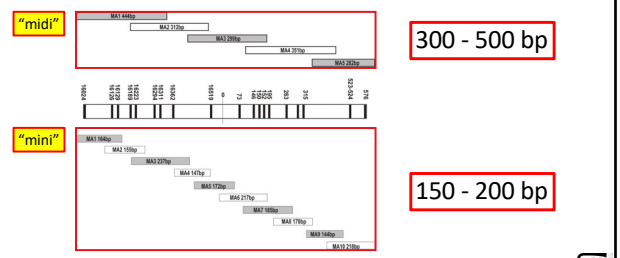
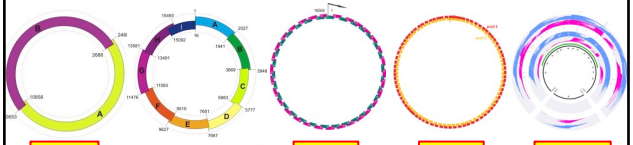
Sanger CE protocols for mtDNA



Berger and Parson 2009

45

mtDNA amplification/enrichment strategies



Fendt et al 2009	GMI modified	Parson et al 2015	Precision-ID (TFS)	Eduardoff et al 2017
mtG ca. 8.5 kbp	mtG ca.2 kbp	CR/mtG ca. 300 bp	CR/mtG ca. 175 bp	PEC CR/mtG < 70 bp

PGM | Ion Chef/Ion S5 | MiSeq FGx®

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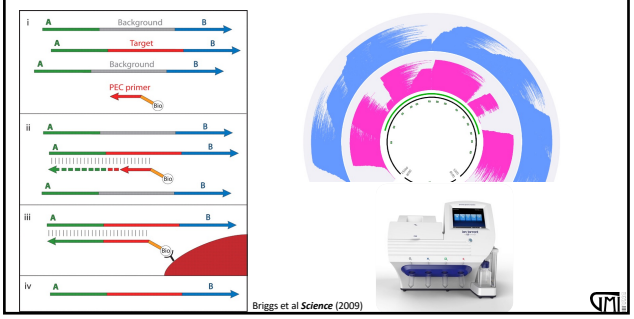
Primer Extension Capture mtDNA CR MPS



Briggs et al *Science* (2009)

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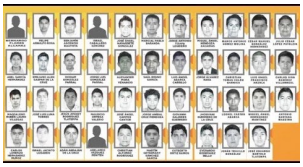
SEPTEMBER 26, 2014, IGUALA, MEXICO

43 male students from the Ayotzinapa Rural Teachers College went missing

Bag was found in a **water** with putative human remains

We received **17 severely burnt** fragmented remains



REUTERS EDITION US

HOME BUSINESS MARKETS WORLD POLITICS TECH OPINION BREAKINGVIEWS

Austrian forensic experts may shed light on Mexico massacre

BY SHADIA NASRALLA

VIENNA | Mon Nov 10, 2014 12:07pm EST

Television cameramen walk at a garbage dump where remains were found outside the mountain town of Cocula, near Iguala in the southwestern state of Guerrero, November 8, 2014.

CREDIT: REUTERS/HENRY ROMERO

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Conventional DNA analysis

17 samples cleaned and DNA extracted (Phenol/Chloroform)

qPCR nDNA (Niederstätter et al 2007) brought promising quants in only 1 sample

This sample gave a full aSTR profile matching 1 of the 43 families

qPCR mtDNA (Niederstätter et al 2007) brought no quants in the remaining 16 samples (143 bp)

The New York Times

AMERICAS

Remains of Student in Mexico Identified

By RANDAL C. ARCHIBOLD and PAULINA VILLEGAS DEC. 6, 2014

Ezequiel Mora, father of Alexander Mora, whose remains were said to have been identified, mourns at his home in El Porvenir, Mexico, on Sunday.

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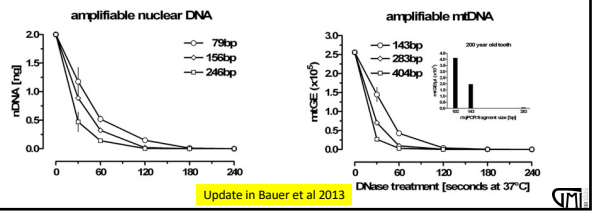
Quantitation of mtDNA

Modular system (Niederstätter et al 2007)

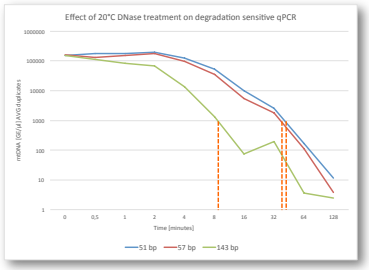
- parallel determination of nDNA (Alu Yb8) and mtDNA
- or nDNA/mtDNA quantitation with internal PCR control (IPC)
- inhibition and degradation sensitive



Update in Bauer et al 2013

50

qPCR values of DNase-degraded mtDNA



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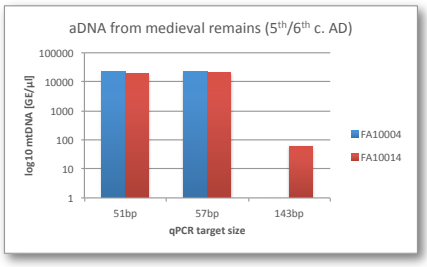
qPCR values of DNase-degraded mtDNA



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Application of the PEC method to Mexican remains

Rationale for approaching the case at all:

Unknown samples expected to belong to Native American phylogeny (hgs A2, B2, C1, D1, D4 and sublineages)

Our lab is exposed to mtDNA lineages from West Eurasia (hgs H, JT, UK, R*, N*, I, X, W and sublineages)

If contamination occurred, it would be easier to distinguish from authentic result

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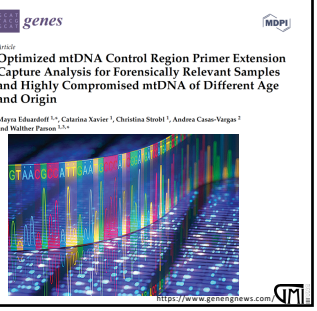
9 of 16 “hopeless” samples brought PEC results



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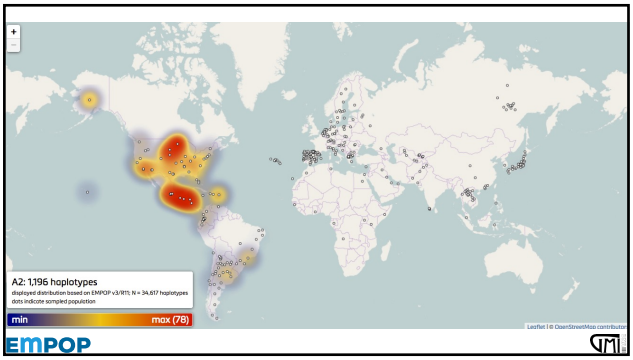

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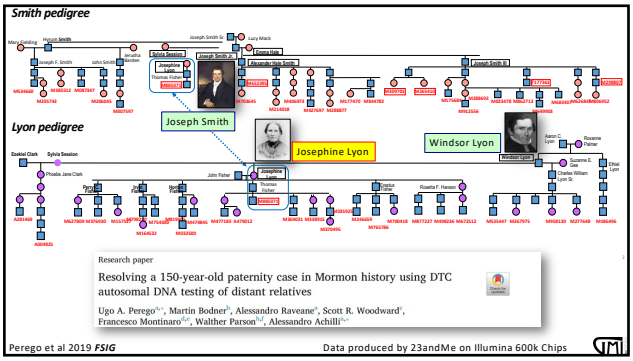

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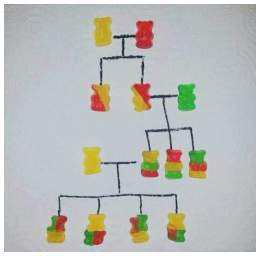
Gummy bears Genetics



IBD - Identical by Descent
IBS - Identical by State

https://9gag.com/gag/aLD1ddg

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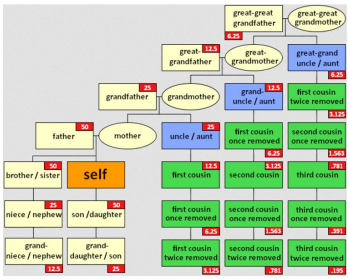
Genetic Genealogy



http://www.genie1.com.au/blog/62-atdna

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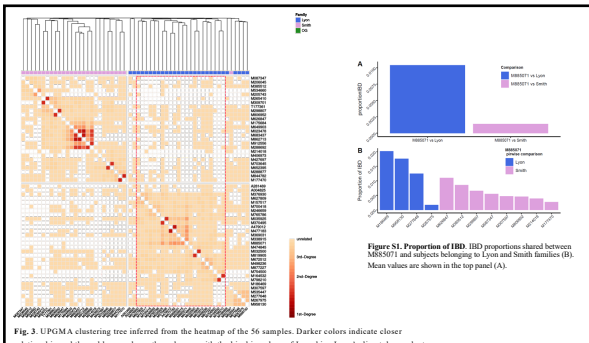

Fig. 3. UPGMA clustering tree inferred from the heatmap of the 56 samples. Darker colors indicate closer relationship and the red box encloses the columns with the kinship values of Josephine Lyon's direct descendants.

Figure S1. Proportion of IBD. IBD proportions shared between MS85071 and subjects belonging to Lyon and Smith families (B). Mean values are shown in the top panel (A).

Perego et al 2019 *FSIG*

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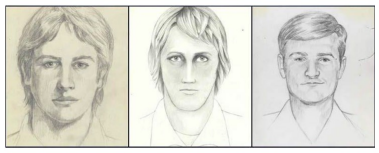
The Case of the „Golden State Killer“ 1974 - 1986

- 13+ murdered, 50+ raped, 120+ burglarized



?

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The Case of the „Golden State Killer“ 1974 - 1986

- The investigation never stopped …
  - Numerous suspects had been identified and excluded
- The investigator
  - Paul Holes (Contra Costa County district attorney's office) – close to retirement
- The DNA sample
  - An unused frozen rape kit from 1980 was analyzed, compared to the CODIS profile from the unknown rapist for authentication
  - Submitted to commercial provider for genome-wide SNP analysis, then uploaded to public GEDmatch database
- The genealogist
  - Barbara Rae-Venter, retired patent attorney working in genealogy to help adoptees find their parents

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The Case of the „Golden State Killer“ 1974 - 1986

[GED match] **Tools for DNA and Genealogy Research**

GEDmatch provides DNA and genealogical analysis tools for amateur and professional researchers and genealogists. Most tools are free, but we do provide some premium tools for users who wish to help support us with contributions. You will need to upload DNA and / or genealogical (GEDCOM) data to make use of the tools here. Registration requires your name, email and a password of your choice. Click HERE to register.

- Two parental family trees established – of Italian and British origin
- The British side was informative leading to hundreds of potential relatives, connecting a 3rd cousin with a putative great-great-grandfather
- Further searches involved birth records, newspaper clippings, social media profiles and family tree data
- One suspect providing a voluntary sample was excluded

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The Case of the „Golden State Killer“ 1974 - 1986

- 13+ murdered, 50+ raped, 120+ burglarized

- Joseph James DeAngelo, arrested April 24, 2018 in Sacramento, CA
  - Born in 1945, Vietnam veteran, 1971 BSc in criminal justice, police officer 1973-1980 in Sacramento and Exeter, married 1973 & divorced 1991, 3 daughters, living since then in Citrus Heights, Sacramento

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The Case of the „Golden State Killer“

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The future of Forensic Genetics

1985-1995	Exploration
1995-2005	Harmonization
2005-2015	Growth
2015-	Sophistication

adapted from Butler JM ***Phil. Trans. R. Soc. B*** (2015)

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Paradigm Shift in Forensic Genetics

Identification - Prediction
Genetics - Genomics

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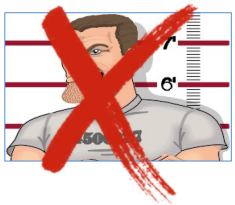
Cases without suspects



Provide investigative leads through DNA

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the invisible gorilla

Ca. 1 min movie of two teams playing basketball
Q: How often do the teams pass a ball

A lady in a gorilla costume walks through

228 test persons watching the movie

Results
194 counted the passes correctly
In most groups ca. **50%** of the participants did not report seeing the gorilla
Success **higher** in participants who counted the black team

= **Inattentual blindness**

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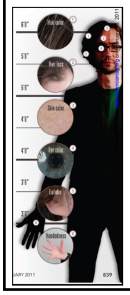
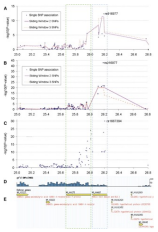
Forensic DNA Phenotyping





OCA2/HERC2

Kayser et al ***AJHG*** 2008

Kayser et al ***FSIG*** 2015

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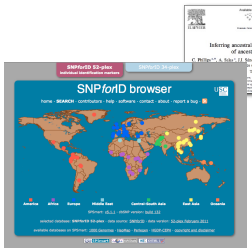
Ancestry Informative DNA Markers

The Madrid bomb investigation

SNPs



Slide kindly provided by C. Phillips, USC Spain

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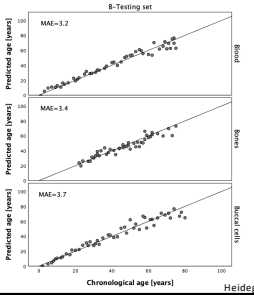
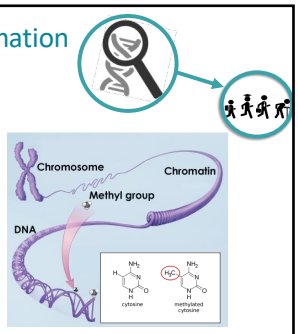
Age estimation





Heidegger et al 2022

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Forensic Genomics VU1 (planned June 2024)

Forensic Genomics (Wahlfach) - 24S - VU

Dashboard / Meine Kurse / Forensic Genomics (Wahlfach) - 24S - VU

Appearance
HIrisplex-S
Erasmus MC Genetic Identification
Indiana Purdue University Walsh Lab Genetic Tools

Ancestry
University Santiago Compostela (Spain), PCA, SNIPPER
STRUCTURE/CLUMPAC, mtDNA

Age
Somatic panel and model for blood

Sample A

Marker	Y-value
1	96,05
2	58,32
3	45,30
4	0,45
5	30,59
6	17,56

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