Tracing Maternal Roots with Mitochondrial DNA

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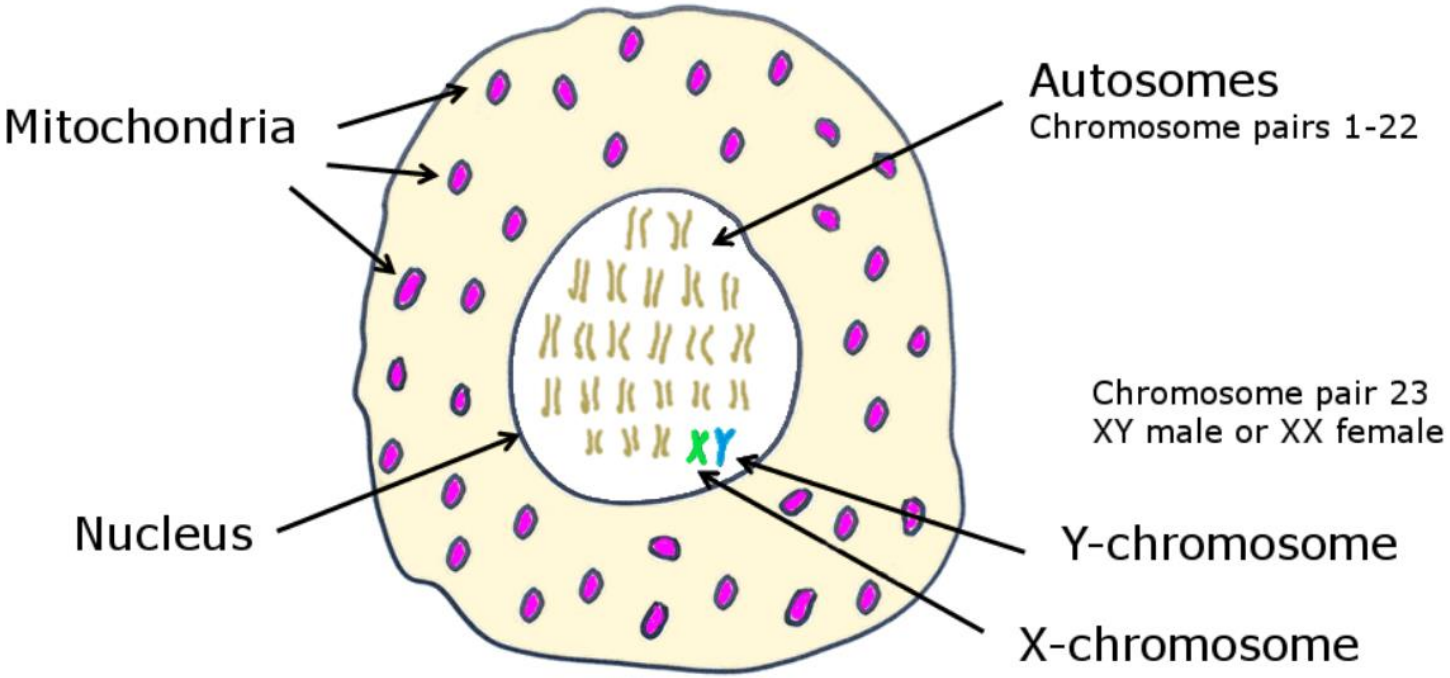
Outline

- Mitochondria and the DNA associated with it?
- Inheritance of mitochondrial DNA (mt-DNA)
- How is it tested?
- Results and terminology
  - Haplogroups and ancient migration routes
  - mt-DNA matches and genetic distance
- Case studies

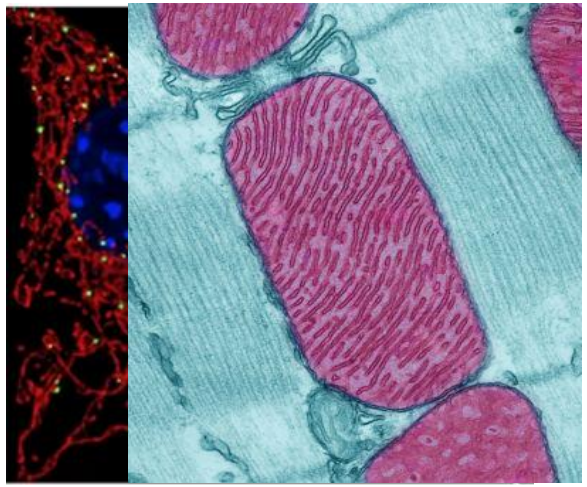
What are the different types of DNA?

Every cell has multiple copies of mtDNA, extranuclear location.

Mitochondria), from Greek *mítos* "length of thread, cord used to separate warp threads" (of uncertain origin) + *chóndrion* "granule"



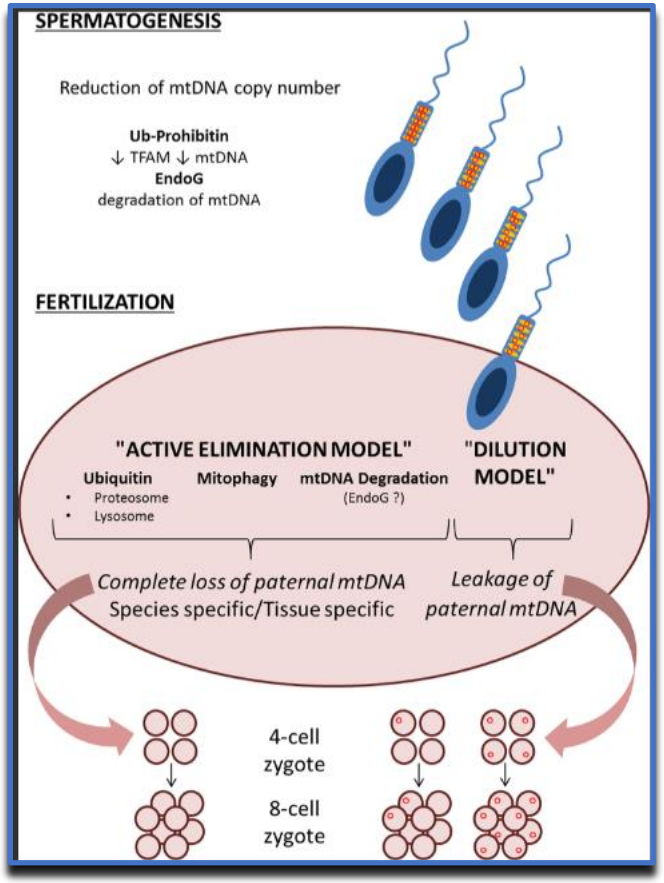
What is Mitochondrial DNA (mt-DNA)?



- **Closed circular dsDNA**
- **Tiny, 5 µm long**
- **100s – 10,000 copies/cell**
  - **2-10 mitogenomes per mitochondria**
- **Exceptions**
  - **e.g., human oocytes: 100,000s**
- **No Recombination (typically a single SNP value per mutation)**

Mitochondrial DNA sequence length is 16,568 base pairs, ~180,000 smaller than total autosomal DNA (haploid); it encodes 37 genes.

Maternal Inheritance
Male mt-DNA eliminated



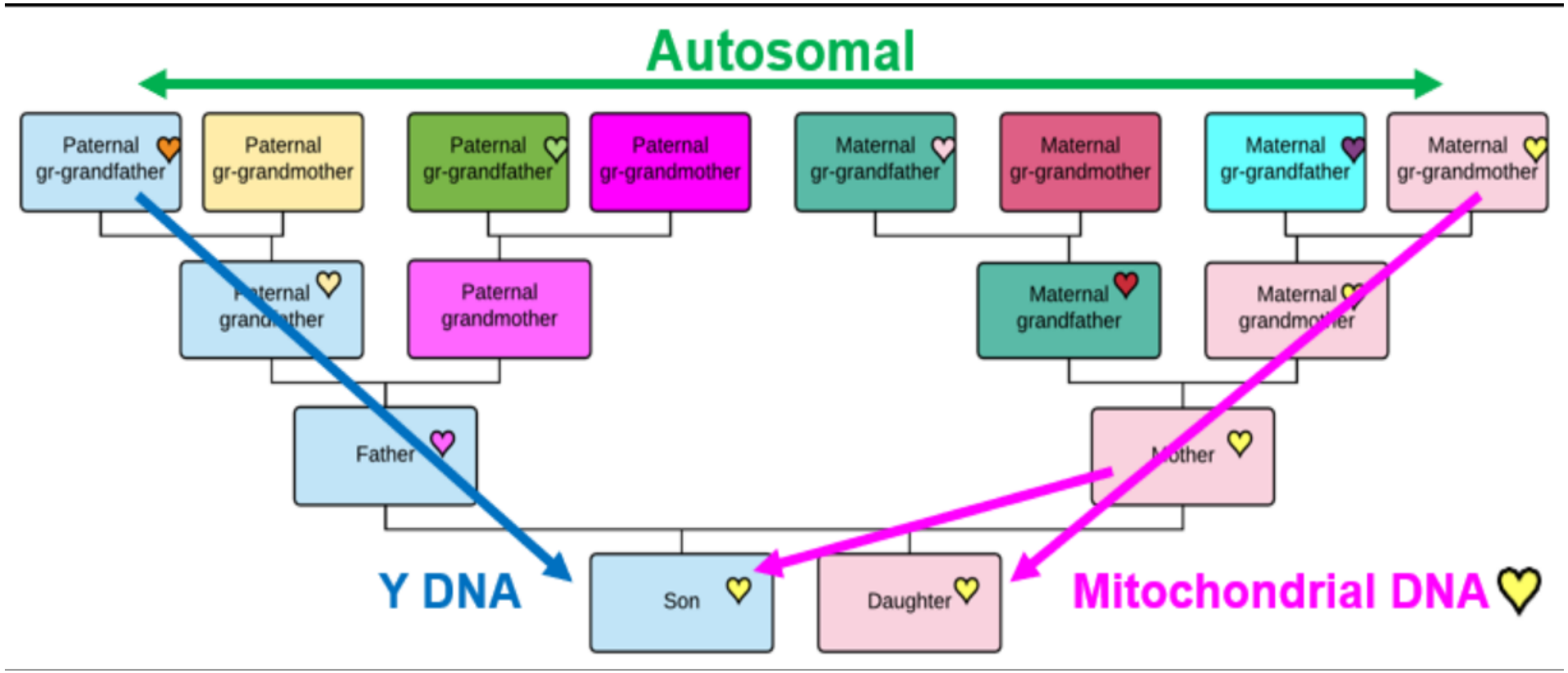
Paul Meier FTDNA YouTube: https://www.youtube.com/watch?v=cpctoeKb0Kw
https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005179

Mito DNA Behaves Different from Nuclear DNA

- Mitochondria evolved over ~2.5 billion years, following the endosymbiosis of a proteobacterial ancestor, which formed the first mitochondrion.
- Diploid human cells contain two copies of each autosomal gene, but many more copies of mtDNA.
- Unlike the nuclear genome, mtDNA is replicated continuously, independent of the cell cycle (relaxed replication), and has a half-life of ~7–10 days depending on the cell type.
- Molecular clock of human mtDNA was calibrated or one mutation every 3624 years over the mitochondrial genome, the amount of mtDNA replication that is required over the life-time of a cell inevitably introduces base substitution errors.
- There is a dramatic reduction in the cellular mtDNA content in early maternal germ line precursors. Results in elimination of minor mutated copies.

Review of Mito DNA heterogeneity: https://www.nature.com/articles/s41576-020-00284-x
mt-DNA mutation rate https://www.cell.com/ajhg/fulltext/S0002-9297(09)00163-3#gr1

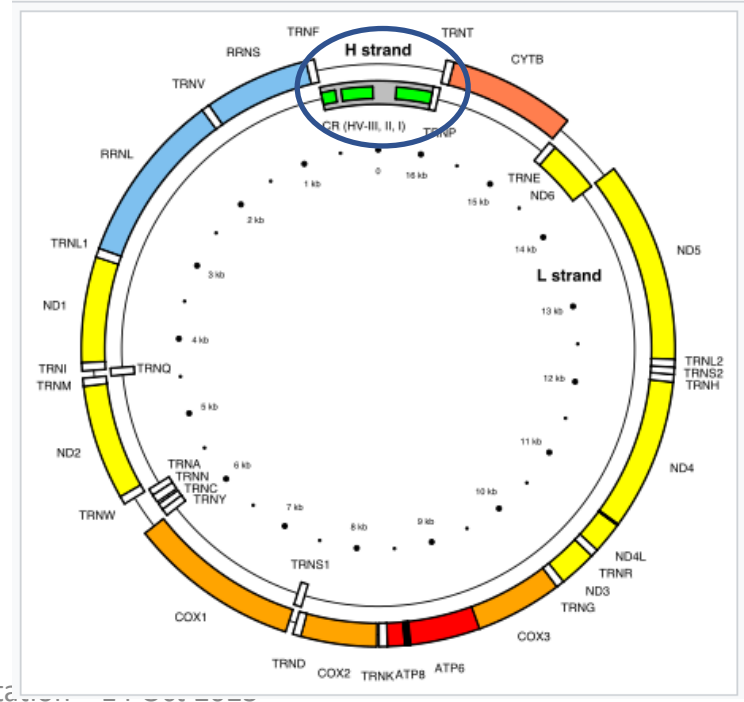
Various Types of DNA Inheritance



Source: Wringing Every Drop Out of Mitochondrial DNA Solving Mitochondrial DNA Puzzles - Roberta Estes

Mitochondrial DNA Structure

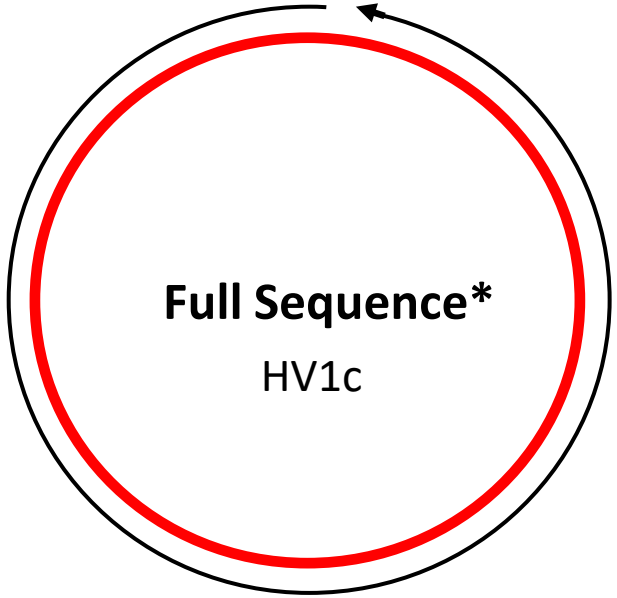
- HVR1 and HVR2 DNA (original test) tests help determine one's **haplogroup**.
- **Homoplasmic** mutations are those which are found in all the copies of mtDNA and **heteroplasmic** mutations are only present in some copies.



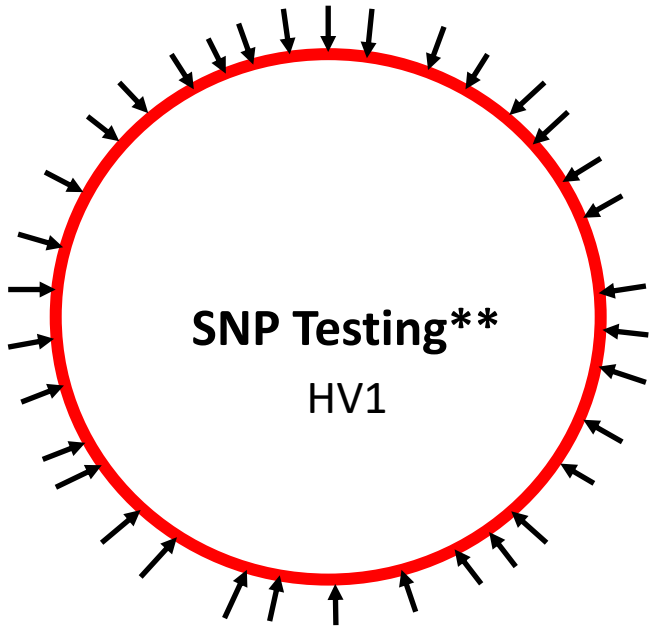
Human mitochondrial genome showing **hypervariable regions** (HVRs) I to III (green boxes) located in the control region (CR; grey box). I: 16,024 to 16,365, II: 73 to 340 ;III (438 to 574)

"…nearly everyone harbours heteroplasmic mtDNA variants obeying two principles: (1) heteroplasmic **single nucleotide variants** tend to arise somatically and accumulate sharply after the age of 70 years, whereas (2) heteroplasmic **indels** are maternally inherited as mixtures with relative levels associated with 42 nuclear loci involved in mtDNA replication, maintenance and novel pathways. These (nuclear) loci may act by conferring a replicative advantage to certain mtDNA alleles." https://pubmed.ncbi.nlm.nih.gov/37587338/

Mito DNA — Two different test types for Mitochondrial DNA



FT DNA performs a complete mtDNA sequence. Yseq DNA and GeneBase offer sequencing of subregions and the complete mito-genome.



23&Me tests 4300 mtDNA SNPs

*Best way to know how closely you're related to a match.
** Cannot be used to find close matches.

Mitochondrial Reference Sequences

Mitochondrial Eve

x

x

x

x

x

x

x

x

x

Haplogroup H

x

x

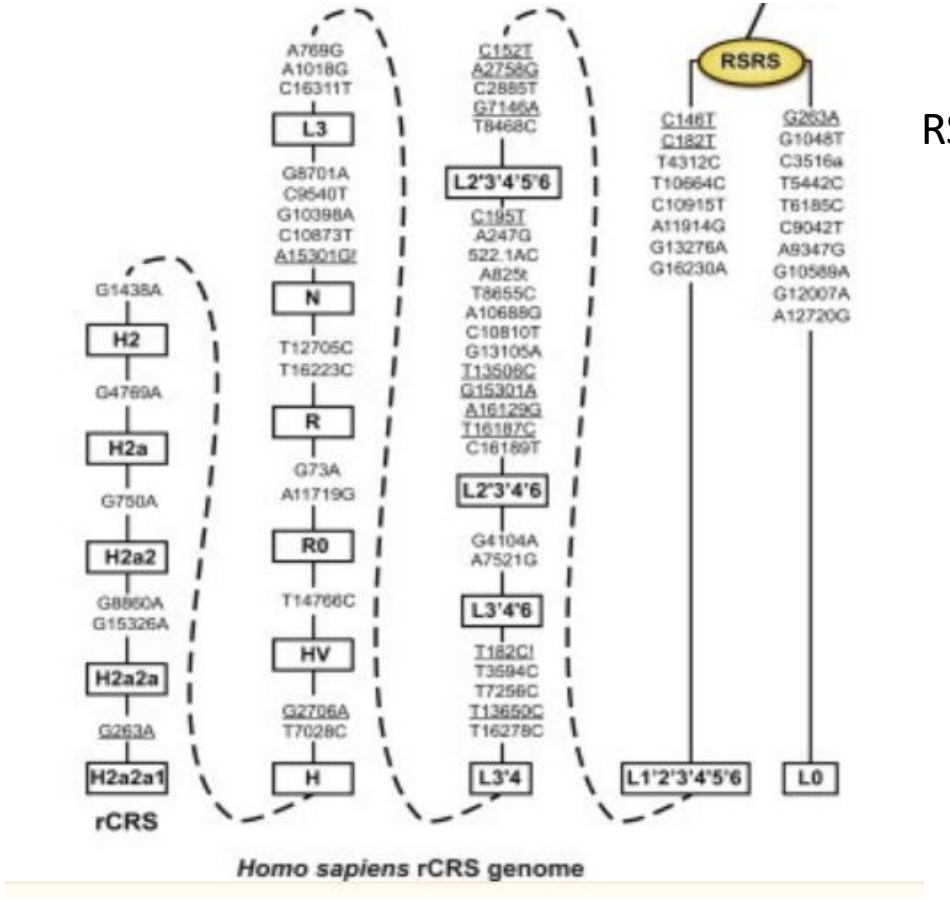
x

rCRS*

x

Haplogroup H tester today

*Cambridge Reference Sequence



Homo sapiens rCRS genome

Mitochondrial "Eve"

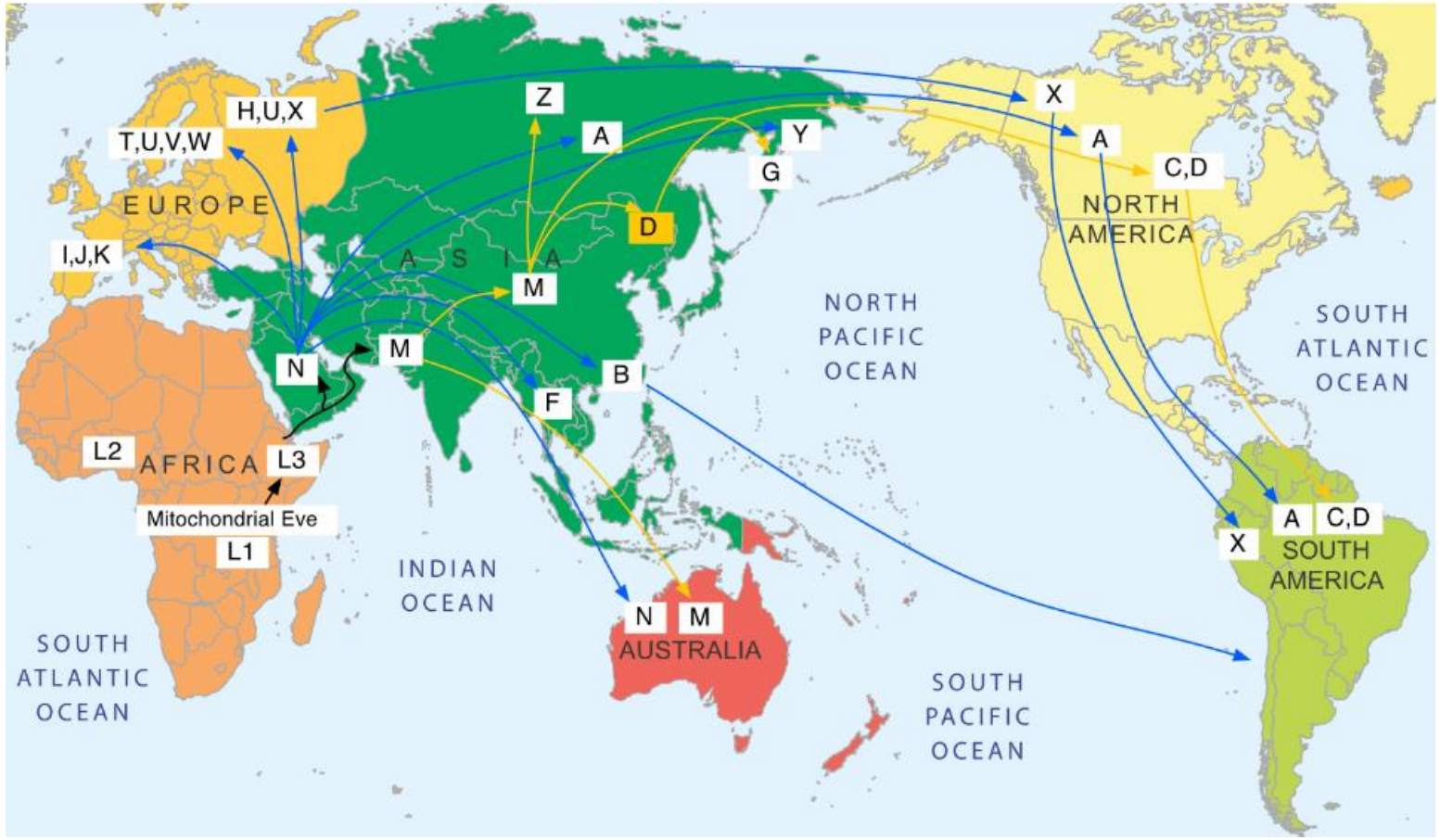
RSRS = Reconstructed Sapiens Reference Sequence

Molecular clock of human mtDNA was calibrated or one mutation every 3624 years over the mitochondrial genome.

Everyone should be being compared directly to Mitochondrial Eve, someone much closer to the root of the mitochondrial phylotree than haplogroup H (when using the *Cambridge Reference Sequence (CRS) first mito DNA sequenced.

Source: DNA eXplained – R. Estes: https://dna-explained.com/2019/05/23/mitochondrial-dna-part-2-what-do-those-numbers-mean/

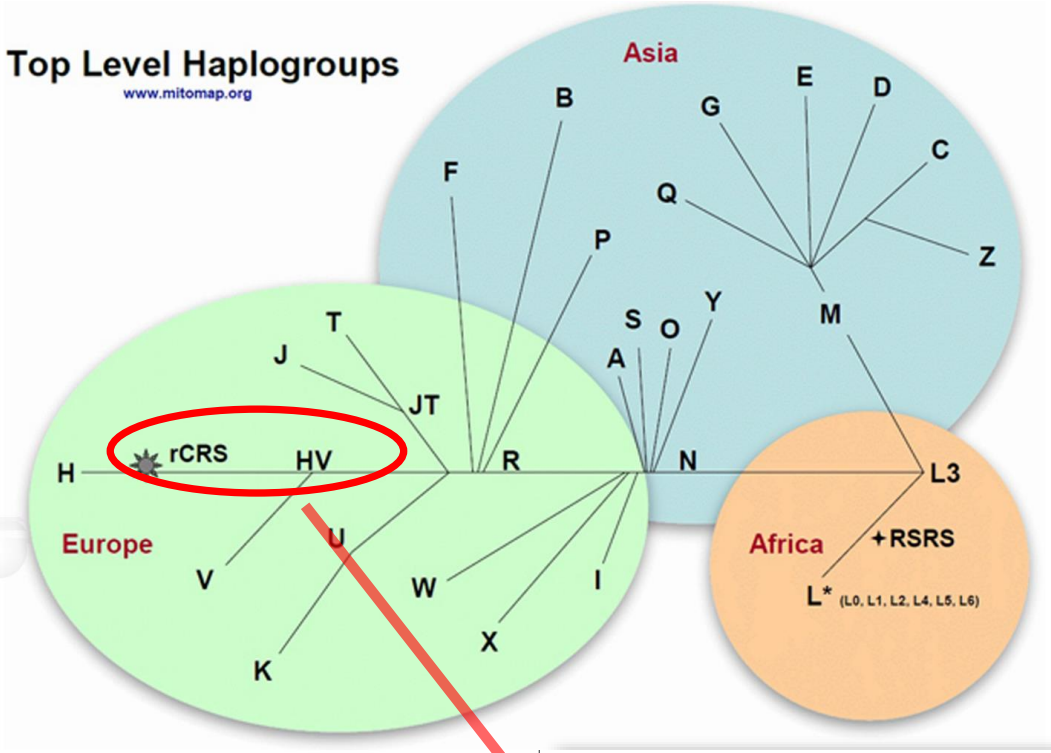
Global Mitochondrial Haplogroup Distributions



Haplogroup letters are not in chronological order based on earliest ancestors.

Source : https://namecensus.com/blog/what-is-the-difference-between-y-dna-and-mtdna-haplogroup-maps/

mt-DNA Results: revised Cambridge Reference Sequence (rCRS)



Revised Cambridge Reference Sequence

HVR1 REFERENCE SEQUENCE		
Show All Positions		
Position	CRS	Your Result
16067	C	T
16362	T	C

HVR2 REFERENCE SEQUENCE		
Show All Positions		
Position	CRS	Your Result
207	G	A
263	A	G
309.1		C
315.1		C

RSRS Values | rCRS Values

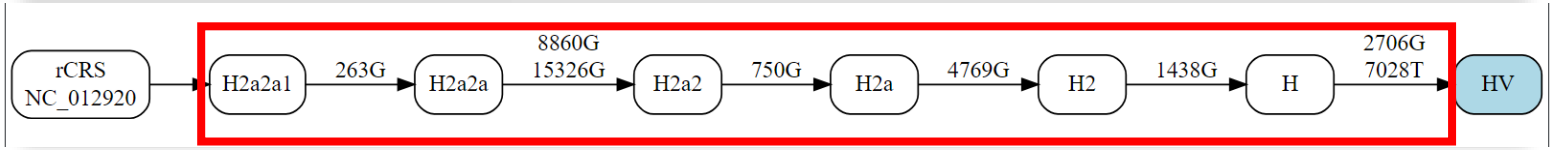
HVR1 DIFFERENCES FROM rCRS	
16067T	16362C

HVR2 DIFFERENCES FROM rCRS			
207A	263G	309.1C	315.1C

CODING REGION DIFFERENCES FROM rCRS				
750G	1438G	2706G	4769G	7028T
7930G	8014T	8020A	8860G	13933G
15218G	15326G			

HV1

HV1c



http://www.phylotree.org./resources/RSRS_annotated.htm

Haplotree: https://www.mitomap.org/MITOMAP

Mt-DNA Results Compared to a Second Reference Sequence

Second tab shows the exact position of your differences from the Reconstructed Sapiens Reference Sequence (RSRS).

Extra Mutations	G207 309 315.1C 522. 522 A7930 G8020.A T16362 C16519
Missing Mutations	

My additional mutations differences from RSRS (other than haplotypic ones)

HVR1 DIFFERENCES FROM RSRS				
C16067T	A16129G	T16187C	C16189T	T16223C
G16230A	T16278C	C16311T	T16362C	C16519T

HVR2 DIFFERENCES FROM RSRS				
G73A	C146T	C152T	C195T	G207A
A247G	309.1C	315.1C	522.1A	522.2C

Haplogroup: HV1c

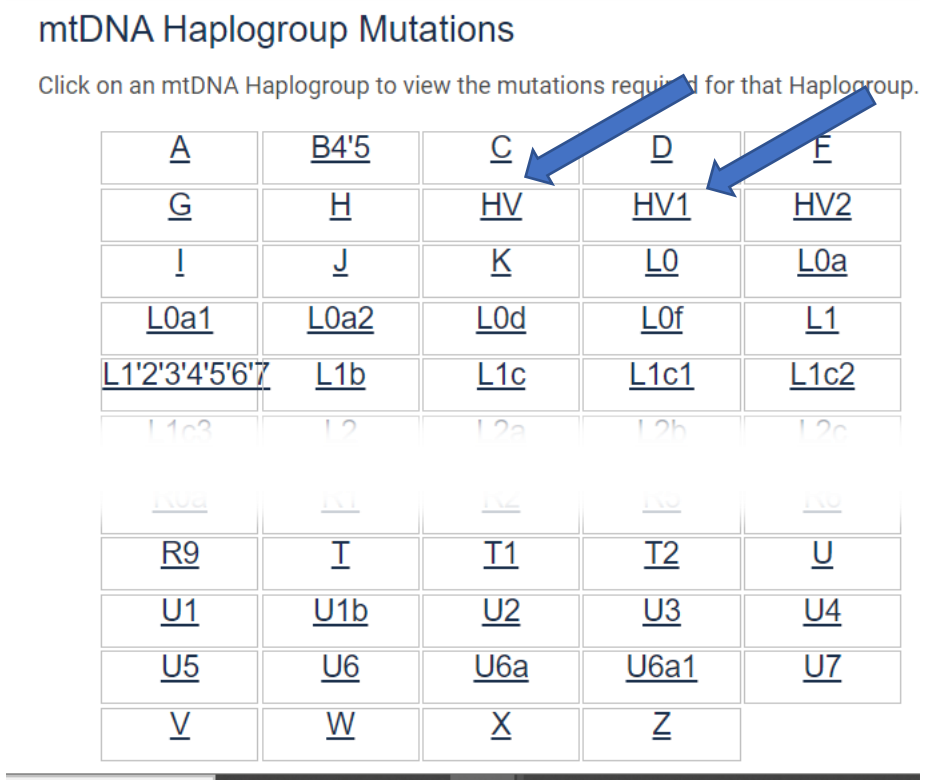
CODING REGION DIFFERENCES FROM RSRS				
A769G	A825t	A1018G	A2758G	C2885T
T3594C	G4104A	T4312C	G7146A	T7256C
A7521G	A7930G	A8014t	G8020A	T8468C
T8655C	G8701A	C9540T	G10398A	T10664C
A10688G	C10810T	C10873T	C10915T	A11719G
A11914G	T12705C	G13105A	G13276A	T13506C
T13650C	A13933G	T14766C	A15218G	

Comparing results to mitochondrial 'Eve' or Reconstructed Sapiens Reference Sequence (RSRS). Eve represents the earliest woman whose haplogroup is the progenitor of all known modern human mt-DNA haplogroups.

HV haplogroup defining SNP: T14766C from the RSRS.
HV1 SNPs: A8014t, C16067T, HV1c: A13933G

HV subclades: https://www.nature.com/articles/s41598-019-48596-1#Tab1
https://www.familytreedna.com/mtdna-haplogroup-mutations.aspx

FT DNA Resources



mtDNA Haplogroup Mutations

Click on an mtDNA Haplogroup to view the mutations required for that Haplogroup.

A	B4'5	C	D	F
G	H	HV	HV1	HV2
I	J	K	L0	L0a
L0a1	L0a2	L0d	L0f	L1
L1'2'3'4'5'6'7	L1b	L1c	L1c1	L1c2
R9	T	T1	T2	U
U1	U1b	U2	U3	U4
U5	U6	U6a	U6a1	U7
V	W	X	Z	

Haplogroup defining mutation(s)

Haplogroup	Required Mutations
HV	T14766C

Haplogroup	Required Mutations
HV1	A8014t, C16067T
HV1a	T8277C
HV1a3a	C14443T
HV1b	T12696C
HV1b3b	A10295G, A10750G, A14161G, T16311C!
HV1c	A13933G

Mutations for each haplogroup: https://www.familytreedna.com/mtdna-haplogroup-mutations.aspx

Mito DNA Possible Mutations:

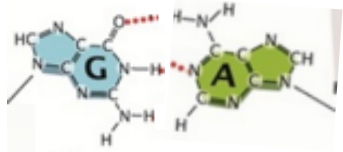
Mutations change one DNA base into a different base, remove or insert a DNA base(s), reverse a DNA base back to origin

- Two Types of point mutations: transitions/transversion
- Insertions
- Deletions
- Reversals

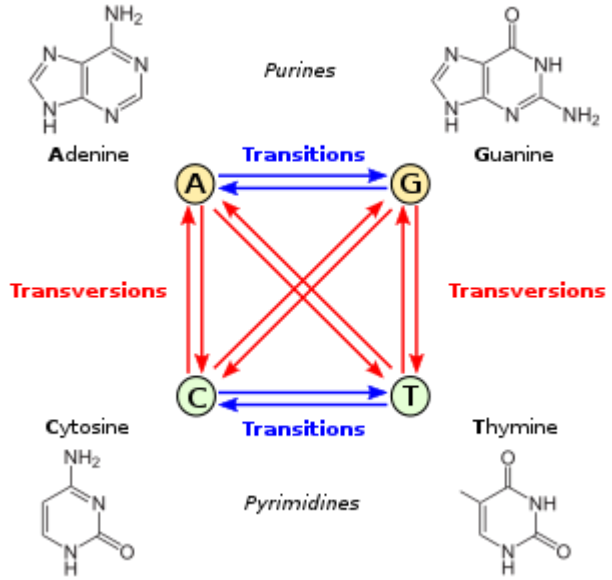
Heteroplasmies (2 results at same position)

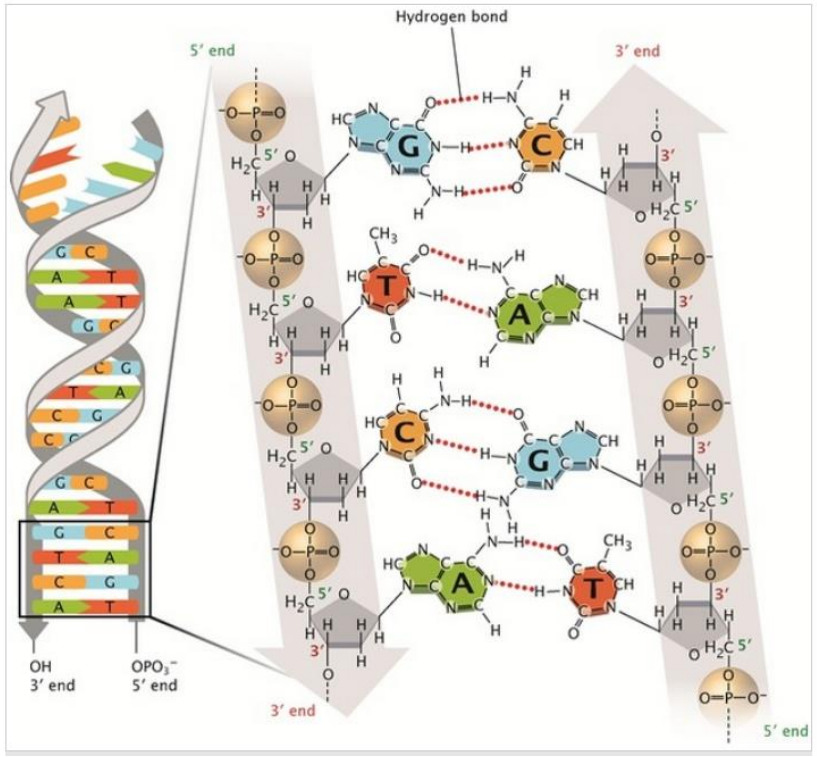
Mito DNA Possible Mutations: Point Mutations

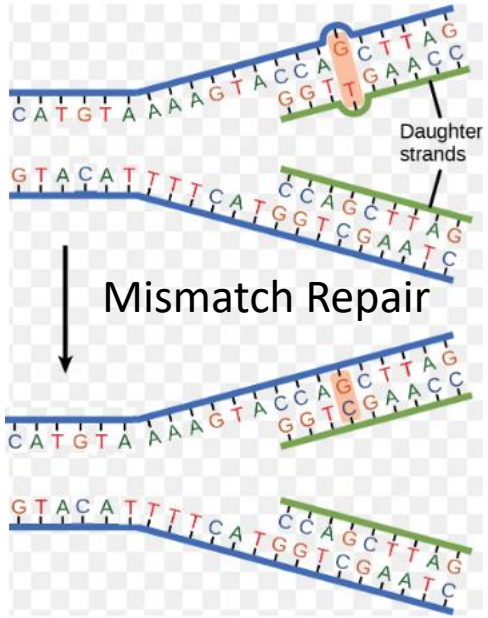
Point mutations change one DNA base into a different base



Two Types of Point mutations







In humans, transitions appear to be about 15 times as frequent as transversions in human mt-DNA.

Mutation introduction: https://help.familytreedna.com/hc/en-us/articles/4404230595855-mtDNA-Mutations-Introduction#accessing-mtdna-mutations-0-0
https://atdbio.com/nucleic-acids-book/Mutagenesis-and-DNA-repair

Naming Convention for Mito-DNA Mutations

- The mt-DNA sequence can undergo mutation changes at any position which could give rise to new haplogroup.
- These mutations can be either a transition or transversion.

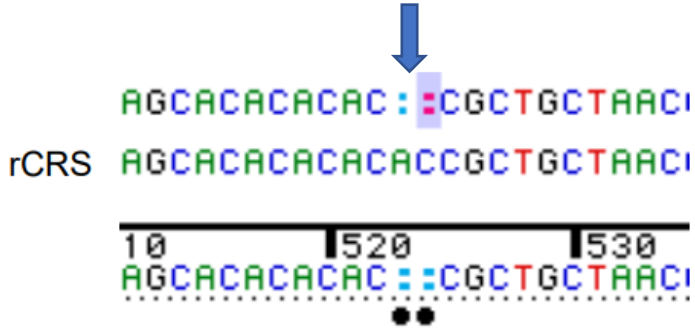
mt-DNA Transition or Transversion

Original Value	Typical Transition Pairing (large trailing letter)	Unusual Transversion Pairing (small trailing letter)
A	G	c or t
T	C	a or g
G	A	c or t
C	T	a or g
Examples:	A8014G	A8014t

Other Mito DNA Possible Mutations: Indels = insertion or deletion

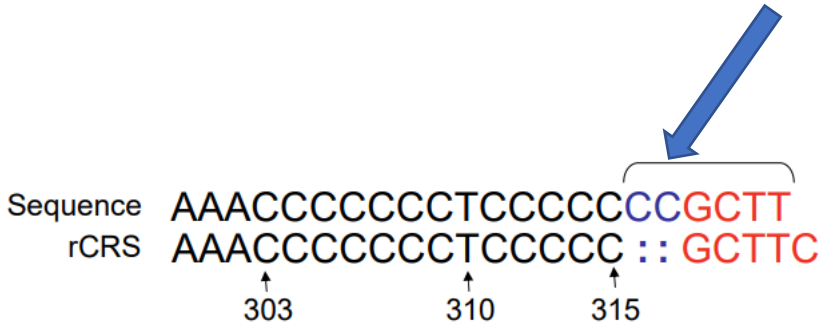
Indel is short for *insertion* or *deletion* of bases within the DNA sequence.

Deletion



523 A-del
524 C-del

Insertion



315.1 C
315.2 C

Type	Text	Meaning
deletion	522- or 522A-del	missing position 522
insertion	315.1C, 315.2C	two extra C's after position 315

Mutation introduction: https://help.familytreedna.com/hc/en-us/articles/4404230595855-mtDNA-Mutations-Introduction#accessing-mtdna-mutations-0-0

Mitochondrial Heteroplasmy

- Sequence differences or mutations are report using single symbols which represent the mutation(s). For transitions or transversions the new nucleotide is reported after the position number e.g., C146T, A8014t. Lower case letters indicate a transversion mutation.

For heteroplasmies, the two values at the position are reported using a single symbol as shown in the table, e.g., C146Y for both a C or T at 146.

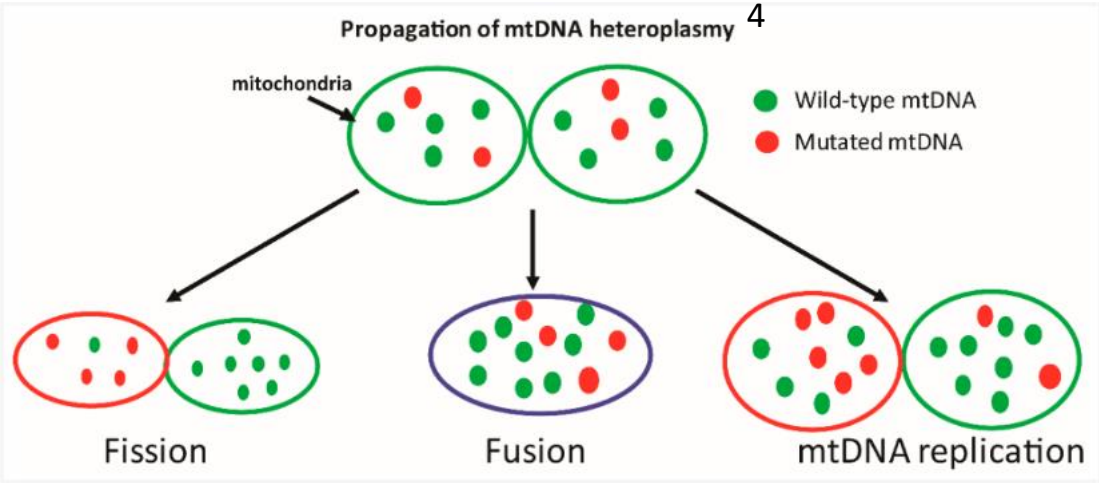
Nucleotide Homo or Heteroplasmy Nomenclature

Symbol	Nucleotide(s)	Symbol	Nucleotide(s)
A	A (Adenine)	T	T (Thymine)
C	C (Cytosine)	G	G (Guanine)
M	A or C	S	C or G
R	A or G	Y	C or T
W	A or T	K	G or T
H	A or C or T	V	G or C or T
D	A or G or T	B	C or G or T
N	G or A or T or C	X	G or A or T or C

Source: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/heteroplasmy

Mitochondrial Heteroplasmy

- Heteroplasmy: presence of 2 or more different mt-DNA genomes within an individual's cells (normal and mutated/variant copies).
- Occurs in at least 10% to 20% of humans.



There are ~ 3 trillion nucleated cells in the human body[(1)]. So, 3 trillion copies of your whole genome which gives possibility of errors in replicating the genome. Human oocytes contain 150,000 copies of mtDNA[(2)]. This could give rise to heteroplasmy if mutations (indels/SNVs) occur in mt-DNA within the germ line. Heteroplasmies accumulates in all tissues[(3)].

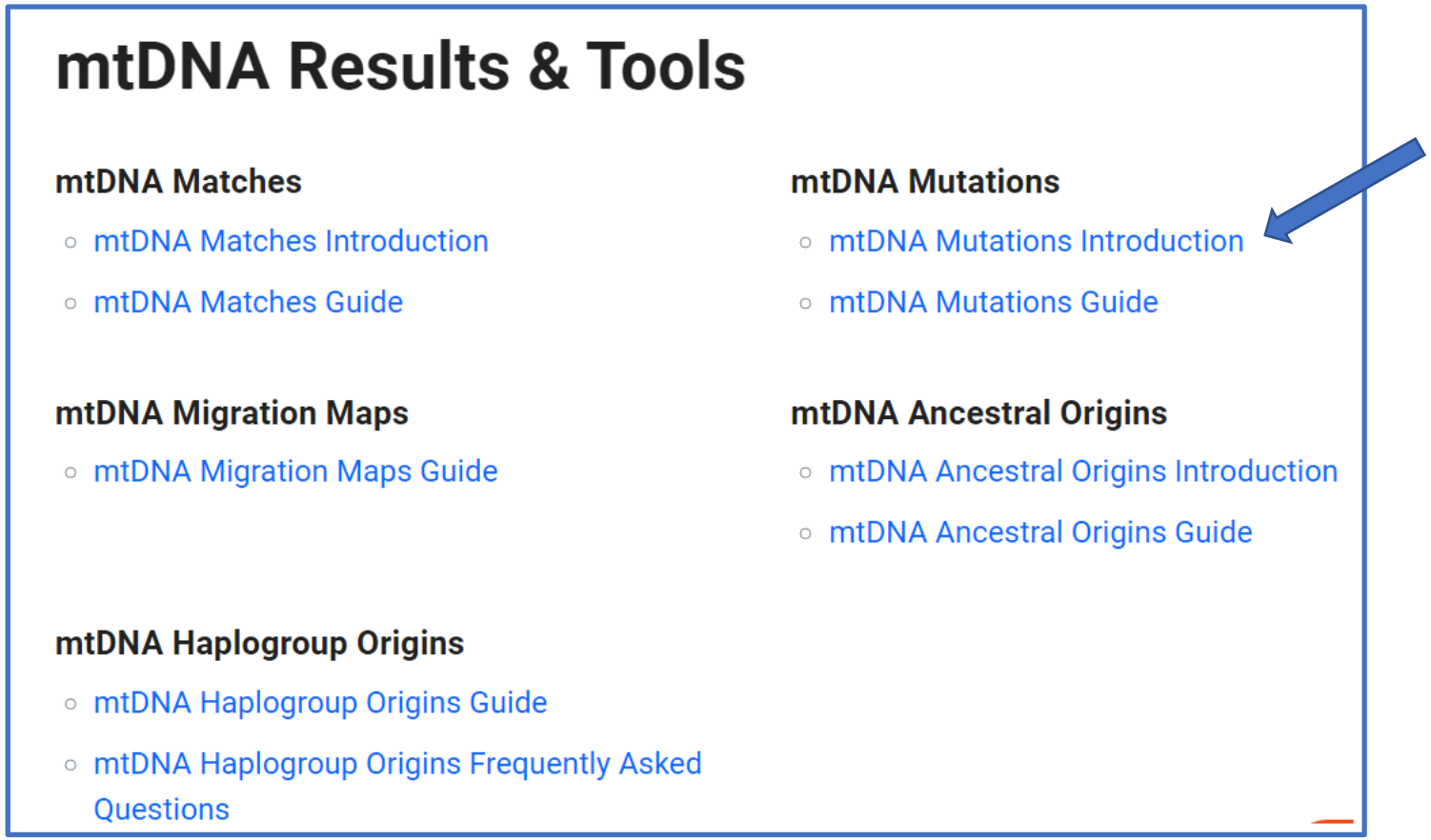
Studies in human show reduction in primordial germ cells which reduces heteroplasmy.

1. https://doi.org/10.1371/journal.pbio.1002533
2. https://doi.org/10.1095%2Fbiolreprod.109.080887
3. Tissue survey: https://www.pnas.org/doi/full/10.1073/pnas.1419651112
4. https://www.mdpi.com/2073-4409/8/2/100

Reduction: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6551220/

Source: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/heteroplasmy

FT DNA Resources



Mutation introduction: https://help.familytreedna.com/hc/en-us/articles/4404230595855-mtDNA-Mutations-Introduction#accessing-mtdna-mutations-0-0

What do the mt-DNA results tell you?

- Determine your haplogroup
  - Shows the geographic region of your maternal line
- Shows your matches
  - Closest mt-DNA relatives
  - These can include people back 100-1000’s years ago
  - Possible eliminate potential ancestors with different haplotypes

Different DNA Inheritance Probabilities

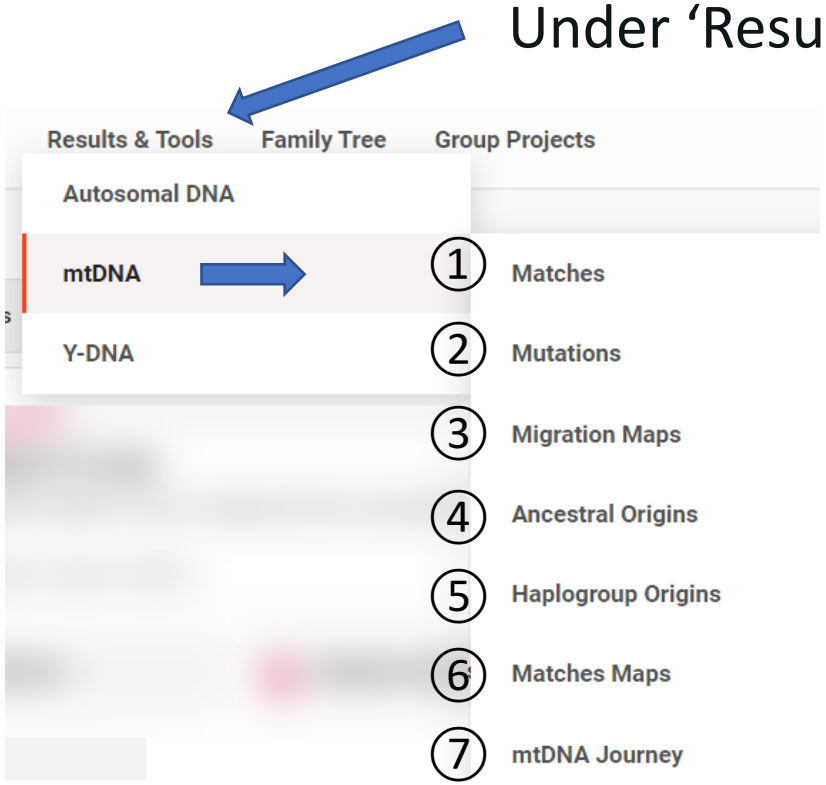
The newest mitoFull test at FTDNA gives a more refined value for the number of generations to common ancestor.

Testing Level	Matching Level	Generations to Common Ancestor	
		50% Confidence Interval	95% Confidence Interval
mtDNA	HVR1	52 (~1,300 years)	NA*
mtDNA Plus	HVR1 & HVR2	28 (~700 years)	NA*
mtFull Sequence	HVR1,HVR2, coding	5 (~125 years)	22 (~550 years)

* The range of generations to a common ancestor at this level is too broad to calculate a 95% confidence period

FamilyTree DNA — Mito DNA Tools

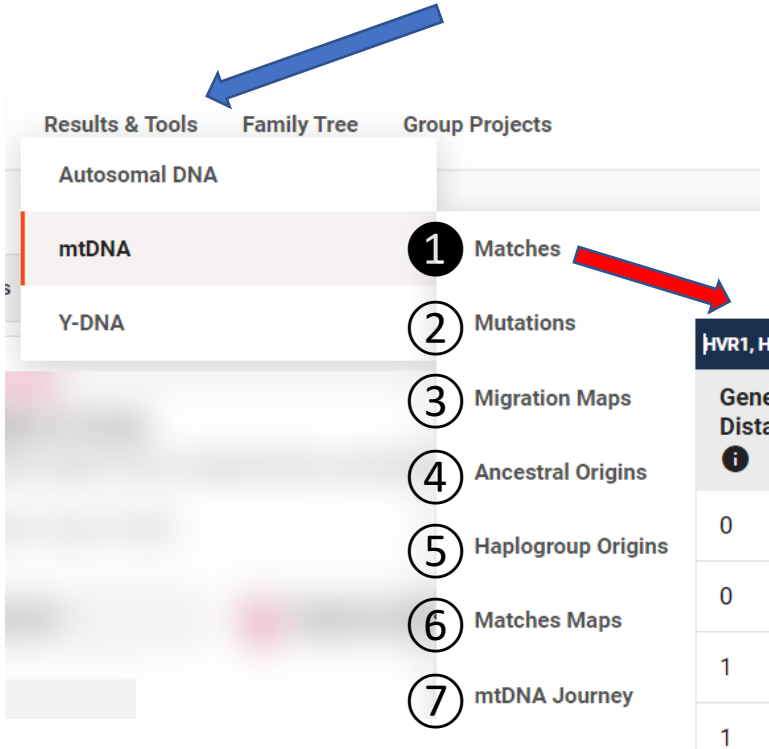
Under ‘Results & Tools’, select mtDNA



Seven mtDNA tools:
Matches, Mutations, Migration Maps. Ancestral Origins, Haplogroup Origins, Matches Maps, mtDNA journey

① Mito Match Tool

Under Results & Tools select mtDNA



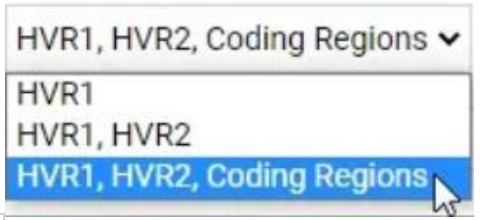
Email link, comment, link to family tree, tests taken by match

HVR1, HVR2, CODING REGIONS - 4 MATCHES

Genetic Distance	Name		Earliest Known Ancestor	mtDNA Haplogroup	Match Date
0	Patricia	FMS FF	Tehan,Fallon,Murray,Garaghty	HV1c	7/29/2022
0	Marjorie	FMS		HV1c	7/29/2022
1	Jonathan	FMS		HV1c	7/29/2022
1	Jocelyn	FMS		HV1c	7/29/2022

Mitochondrial DNA Genetic

Genetic Distance Information and Interpretation



HVR1 GD or # of Mutations Allowed for a Match	HVR1 + HVR2 GD or # of Mutations Allowed for a Match	HVR1 + HVR2, Coding Region GD or # of Mutations Allowed for a Match
0 – no mutations allowed	0 – no mutations allowed	3 mutations allowed

Two high-frequency insertion/deletion locations are completely excluded from difference counts. These are mutations at positions 309 and 315.

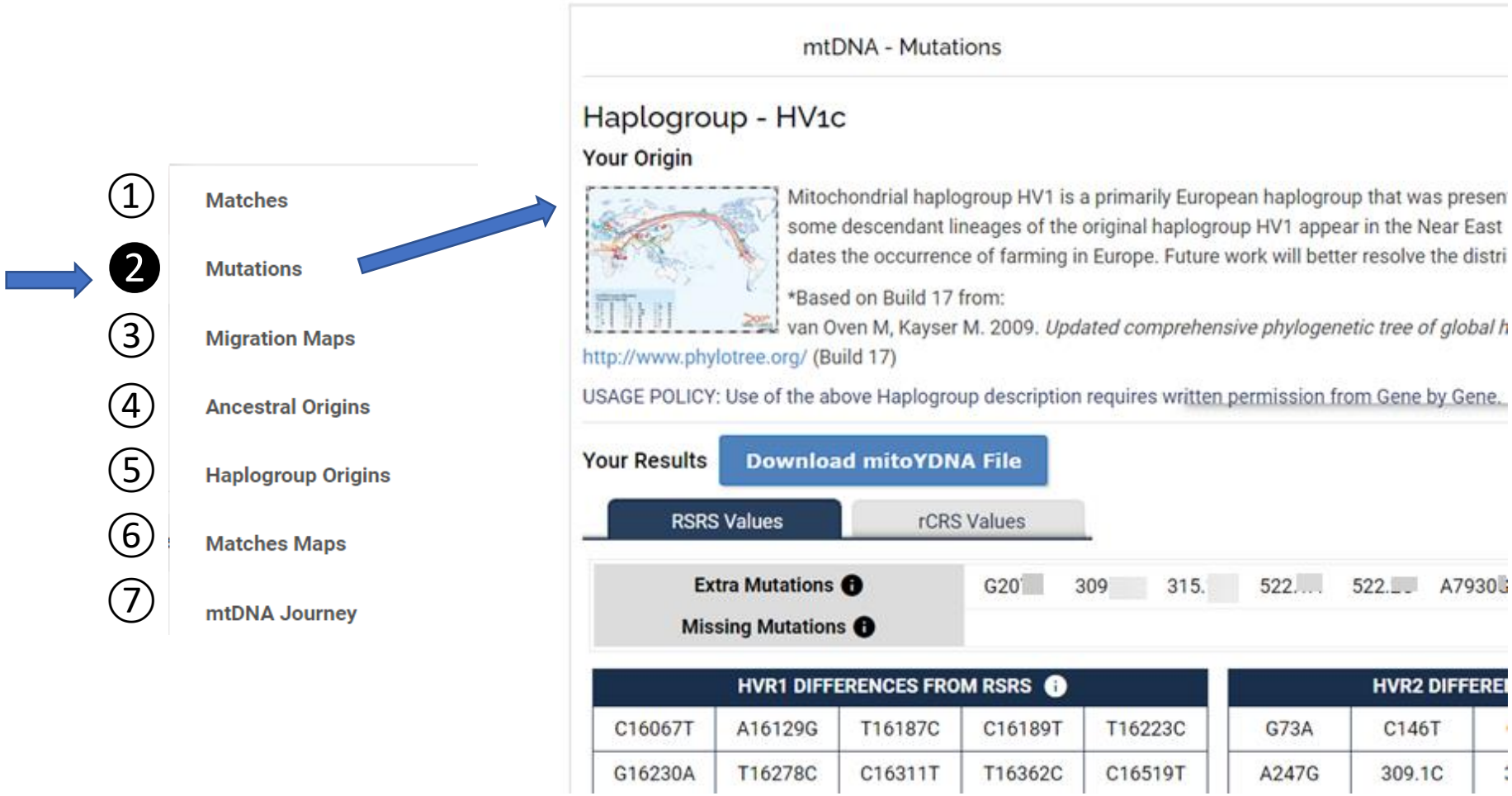
GD Help: https://help.familytreedna.com/hc/en-us/articles/360004684915-mtDNA-Ancestral-Origins-Guide

Mitochondrial DNA Genetic Distance Can Mislead

- Mitochondrial DNA gives a numerical value to how close your matches are to you (GD0, GD1, GD2 or GD3). You are allowed 3 or fewer mismatches to be considered a match
- Usually, their closeness is GD0 > GD1 > GD2 >GD3
- These values can be misleading especially when insertions/deletions in the polyC tract are involved other than at 309 and 315.
- These differences include cases of heteroplasmy (2 different sequence at same position).

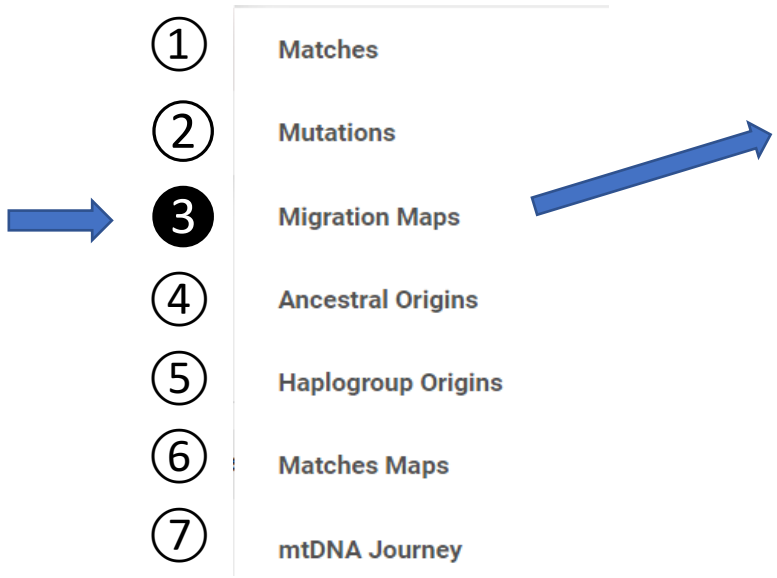
② Mito Matches

Under ‘Results & Tools’, select Mutations



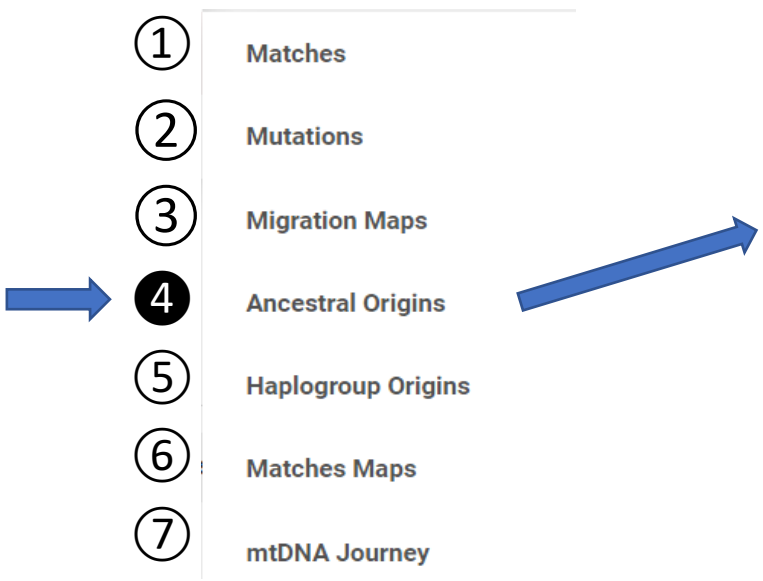
③ Migration Maps

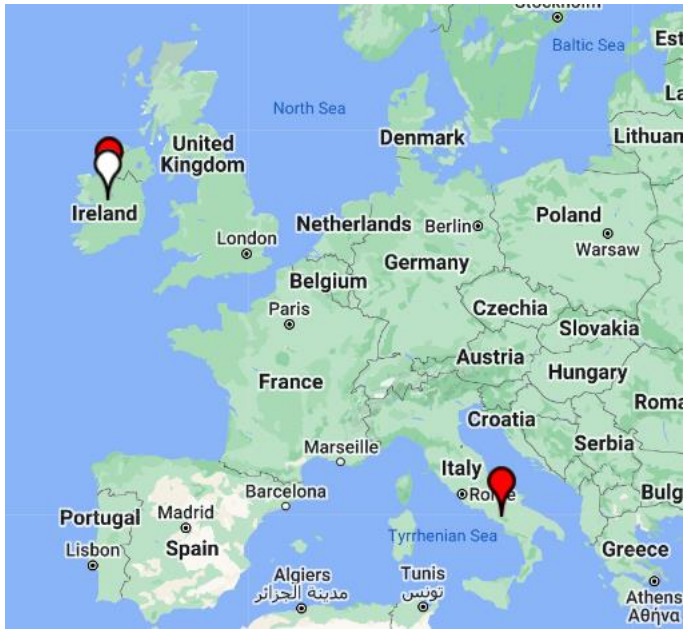
Under ‘Results & Tools’, select Migration Maps



④ Ancestral Origins

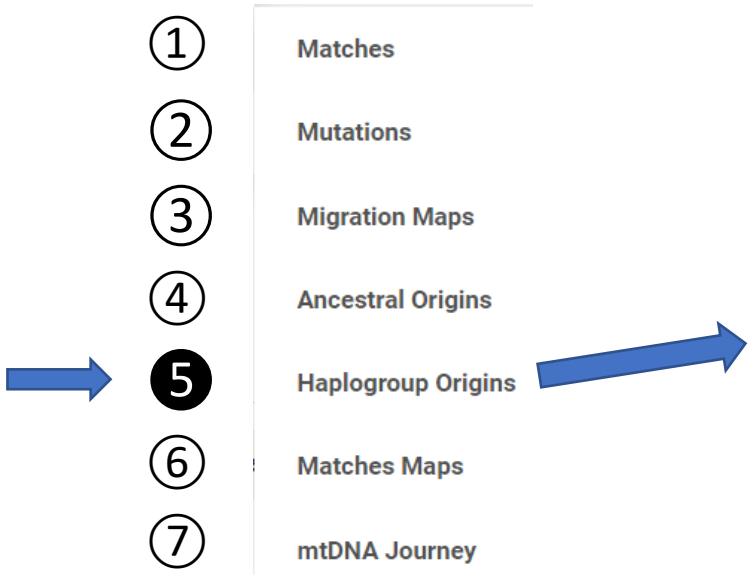
Under ‘Results & Tools’, Ancestral Origins





⑤ Haplogroup Origins

Under ‘Results & Tools’, Haplogroup Origins



Shows country of origin of mtDNA matches

HVR1 MATCHES

Country	Match Total	Country Total	Percentage
England	1	19404	< 0.1 %
Ireland	3	15755	< 0.1 %
Israel	1	416	0.2%
Italy	3	5863	0.1%

HVR1 AND HVR2 MATCHES

Country	Match Total	Country Total	Percentage
England	1	15031	< 0.1 %
Ireland	1	11590	< 0.1 %

HVR1, HVR2, AND CODING REGION MATCHES

EXACT MATCH

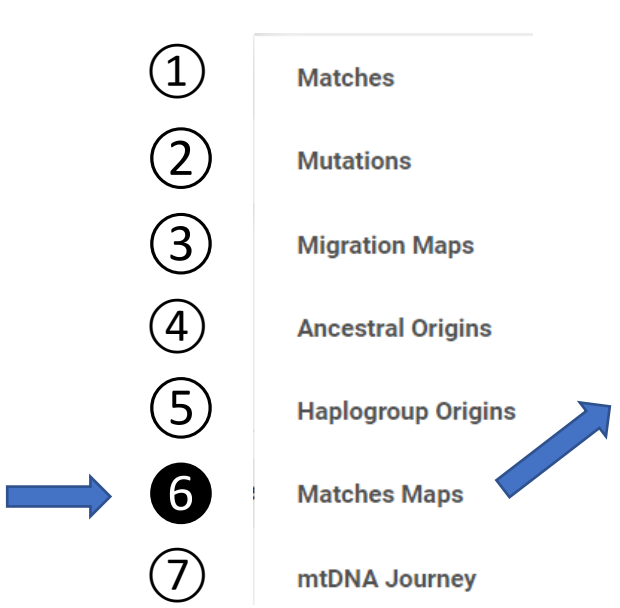
Country	Match Total	Country Total	Percentage
England	1	11350	< 0.1 %
Ireland	1	8957	< 0.1 %

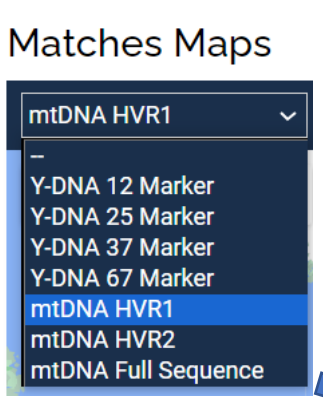
GENETIC DISTANCE -1

Country	Match Total	Country Total	Percentage
Ireland	1	8957	< 0.1 %

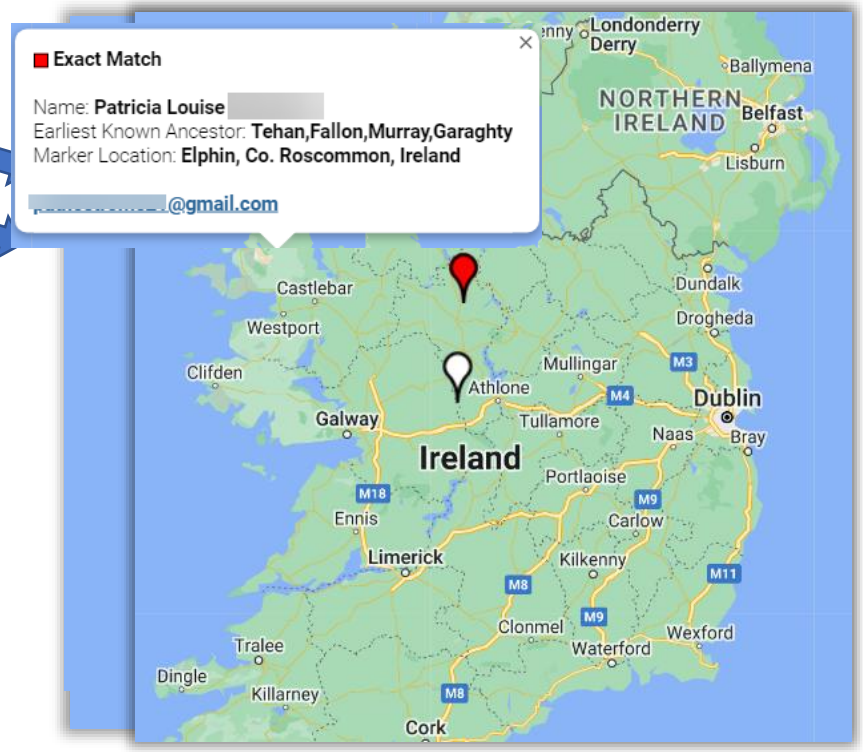
⑥ Matches Maps

Under ‘Results & Tools’, select Match Origins



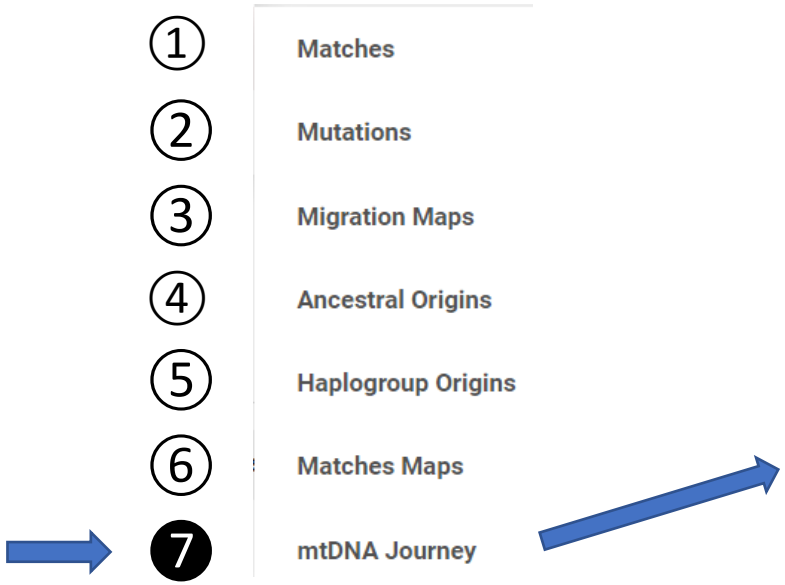


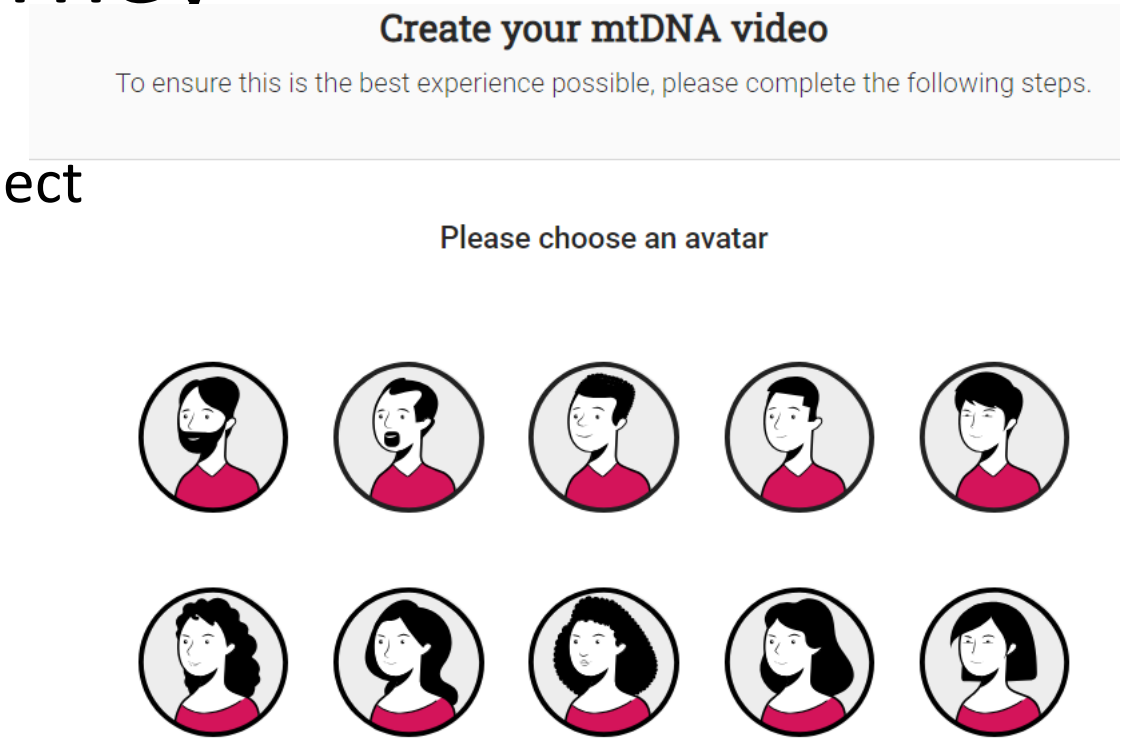
Shows location of closest mtDNA to mtDNA HVR1, HVR2 or Full Sequence matches (or Y-DNA if tested). Select via drop-down menu



⑦ mtDNA Journey

Under ‘Results & Tools’, select mtDNA Journey

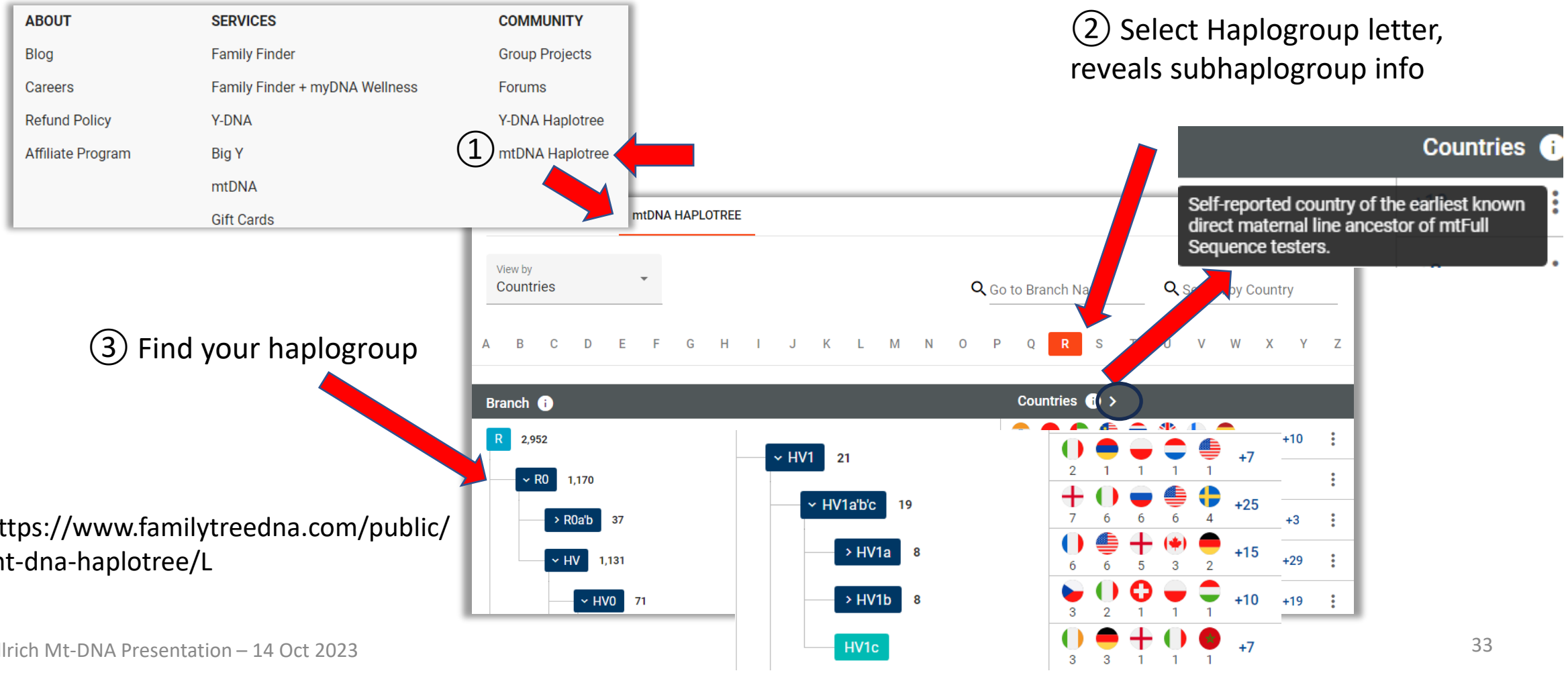




My video:
https://www.familytreedna.com/my/mtdna-journey

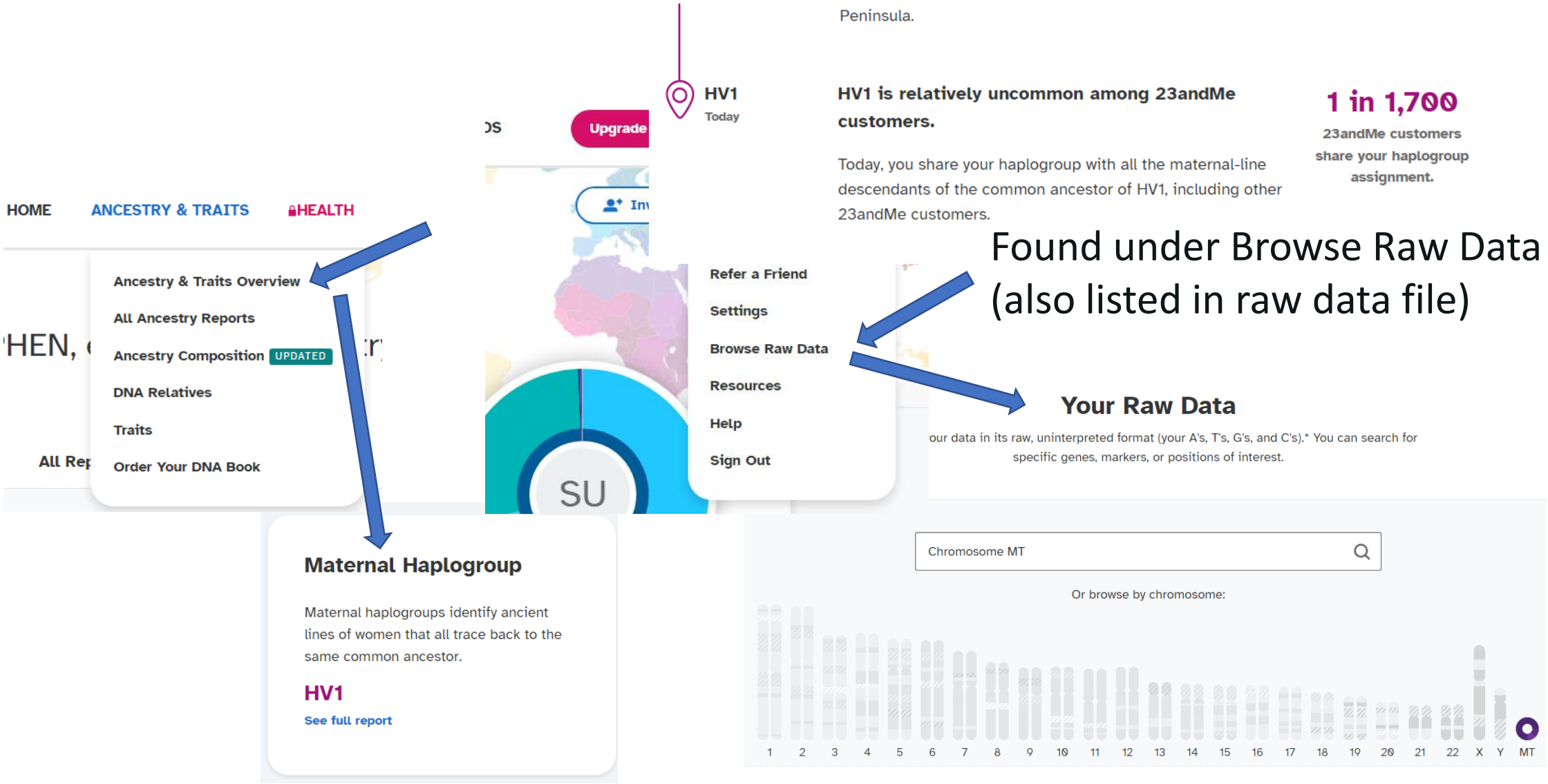
mtDNA Display Haplogroup and Members by Country

Scroll to the bottom of the page to find Community links, select ①



https://www.familytreedna.com/public/mt-dna-haplotree/L

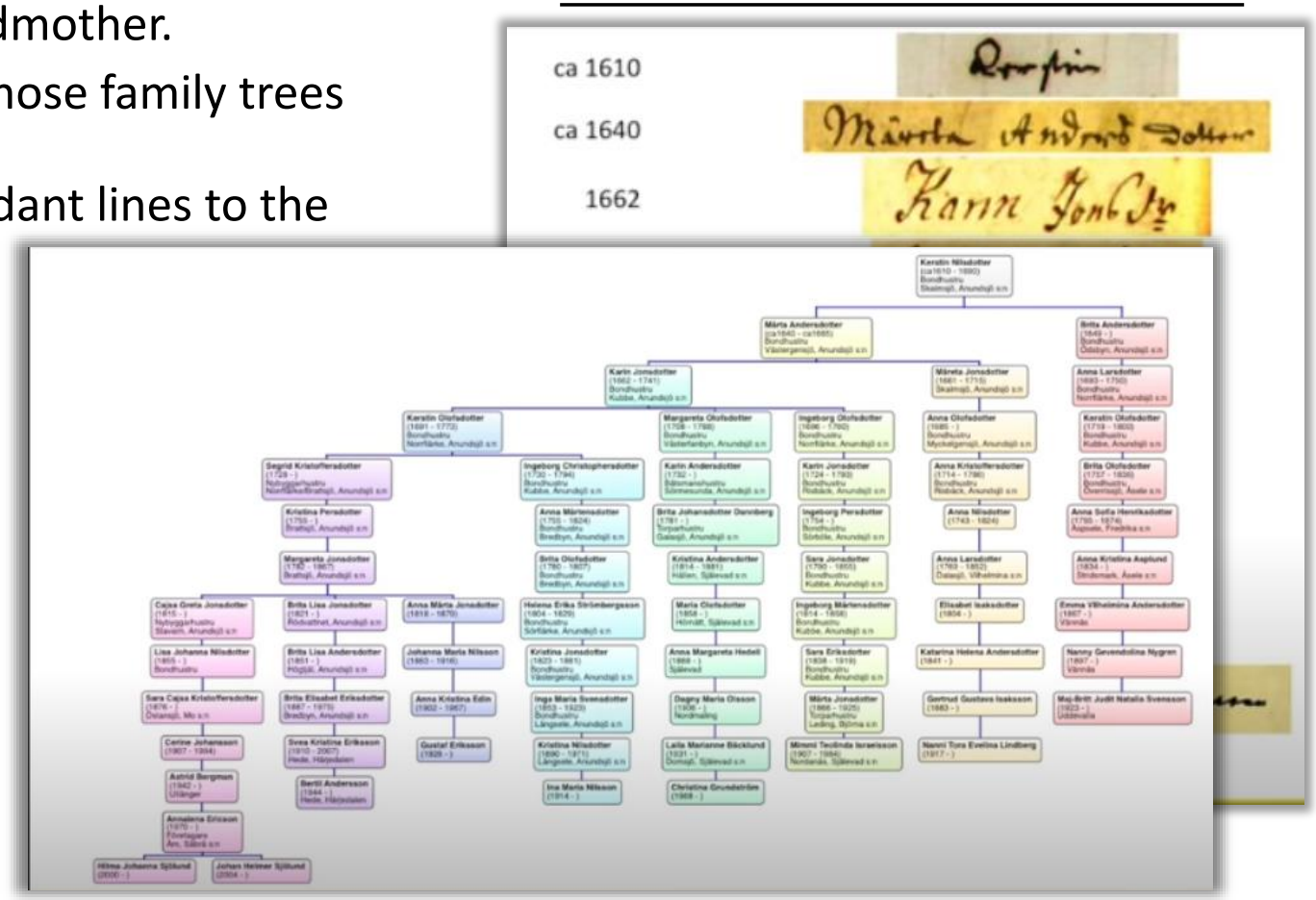
Mito DNA — 23andMe Results



An Example of mt-DNA Success

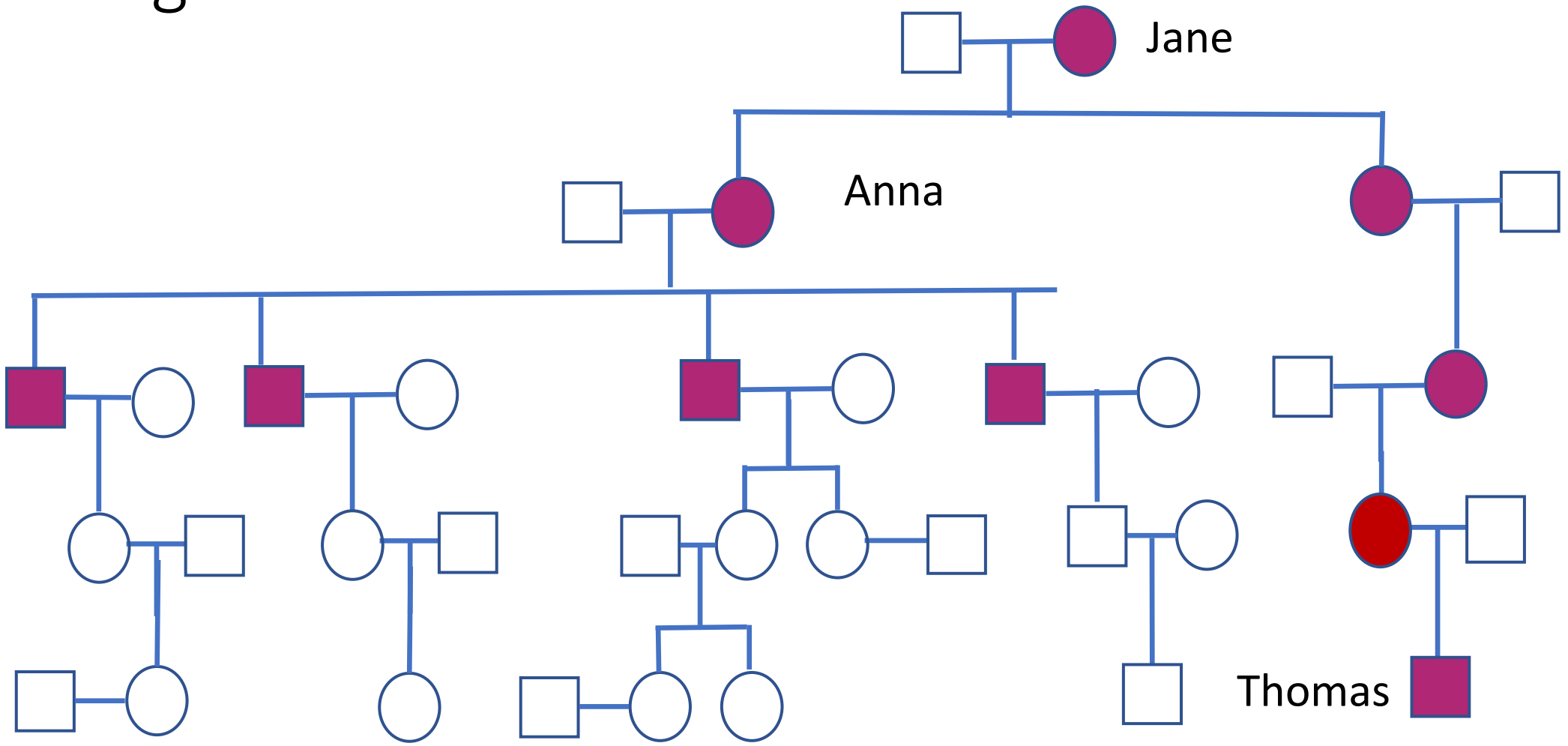
- A Swedish maternal lineage was traced back in the records to ~1610. to a 9th great grandmother.
- Take a mtDNA test found matches whose family trees converged on same area in Sweden.
- Able to link several *maternal* descendant lines to the same ancestor and solve a brickwall.

Maternal Ancestral Line - 11 Gen.



Source: The Power of Mitochondrial DNA – A Swedish perspective - Peter Sjölund (Creative Commons on YouTube): https://www.youtube.com/watch?app=desktop&v=J568xnfNVNw

Option for Finding mt-DNA if your line has a male one generation back



Case Study One: Mito DNA Heteroplasmy

and the Mystery of the fate of Tsar Nicholas and his family

In 1991, a Siberian grave containing nine skeletons thought to be the remains of the last Russian Tsar, Nicholas II, and his family and retinue, were exhumed.

Part of the family was subsequently found in another grave.

Reference: http://www.dnai.org/teacherguide/pdf/reference_romanovs.pdf

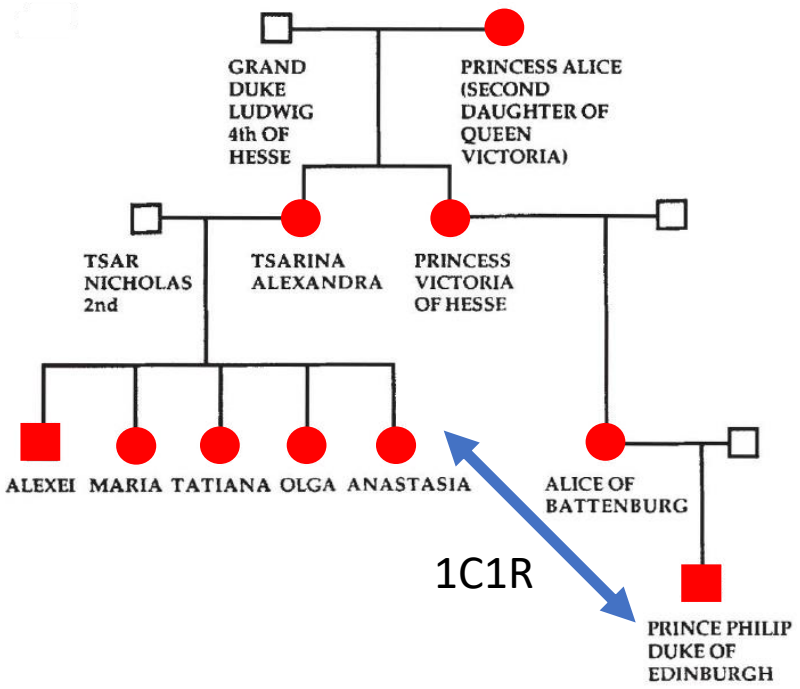
How was the case solved?

- HRH Prince Philip, the Duke of Edinburgh, provided mitochondrial DNA used to identify Tsarina Alexandra and her three daughters.
  - Pedigree chart compared to Elizabeth indicated that Prince Philip is more closely related to Alix than Victoria. One of Philips grandparents was a sibling of Alexandra.
- Two cousins of children of the related to the Tsar's maternal line were found and tested.

https://www.youtube.com/watch?v=FRj4m9Ax9DE

Pedigrees of the Tsar Nicholas and Tsarina Alexandra

The Tsarina's **children** should match descendants in common with their grandmother, Princess Alice.



The **Tsar's** mtDNA should match the mt-DNA descendants of his grandmother, Louisa of Hesse-Kassel.

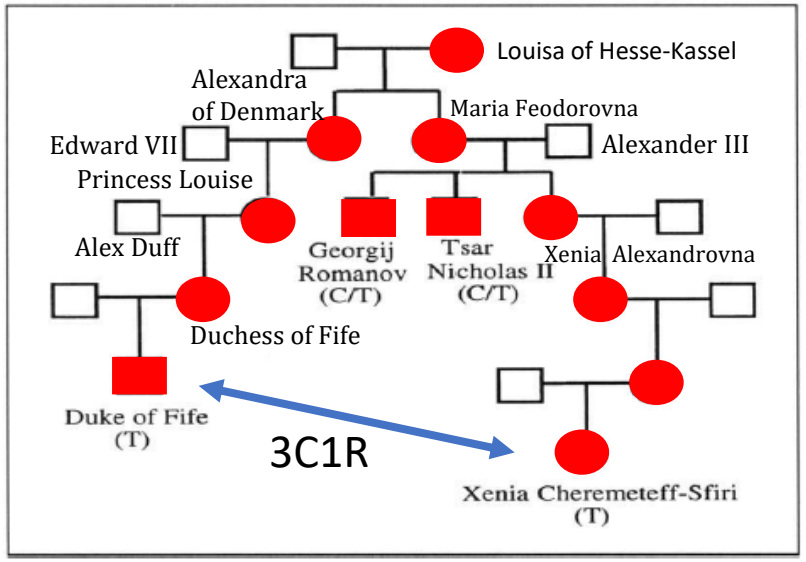


Fig. 3 Lineage of Tsar Nicholas II, indicating individuals whose mtDNA sequences were determined (sequence at position 16169 is listed beneath name). Black symbols indicate the Hessian maternal lineage. D-loop sequences from the Duke of Fife were determined in a previous study, and match those of Countess Xenia Cheremeteff-Sfiri[2].

Results of mt-DNA Analysis to ID Romanov Family Members

		HVR2							HVR1											
	Position	73	146	195.0	263	309.1	309.2	315.1	16,111	16,126	16,169	16,261	16,264	16,278	16,293	16,294	16,296	16,304	16,311	16,357
	Reference Sequence	A	T	T	A	-	-	C	C	T	C	C	C	C	A	C	C	T	T	T
3C1R‡	Tsar Alexander	G	T	T	G	-	-	C	C	C	Y	C	C	C	A	T	T	T	T	T
3C1R‡	Georgij Romanov	G	T	T	G	-	-	C	C	C	Y	C	C	C	A	T	T	T	T	T
3C1R‡	Xenia	G	T	T	G	-	-	C	C	C	C	C	C	C	A	T	T	T	T	T
3C1R‡	Duke of Fife	G	T	T	G	-	-	C	C	C	C	C	C	C	A	T	T	T	T	T
1C1R	Prince Philip	A	T	T	G	-	-	C	T	T	C	C	C	C	A	C	C	T	T	C
1C1R	Tsarina 1	A	T	T	G	-	-	C	T	T	C	C	C	C	A	C	C	T	T	C
1C1R	Tsarina 2	A	T	T	G	-	-	C	T	T	C	C	C	C	A	C	C	T	T	C
1C1R	Tsarina 3	A	T	T	G	-	-	C	T	T	C	C	C	C	A	C	C	T	T	C

(-) = NOT present. **Y = C or T** **A, T, C, G =** **differs from reference.**

‡ Cousins (Xenia and the Duke) have lost the 16,169 C/T heteroplasmy.

The Tsar and his descendants

Louisa of Hesse-Kassel's mt-DNA is source for Tsar Nicholas II Haplogroup T, (73G, 263G, 315.1C, 16126C, 16169Y 16294T, 16296T)

The Tsarina and her descendants

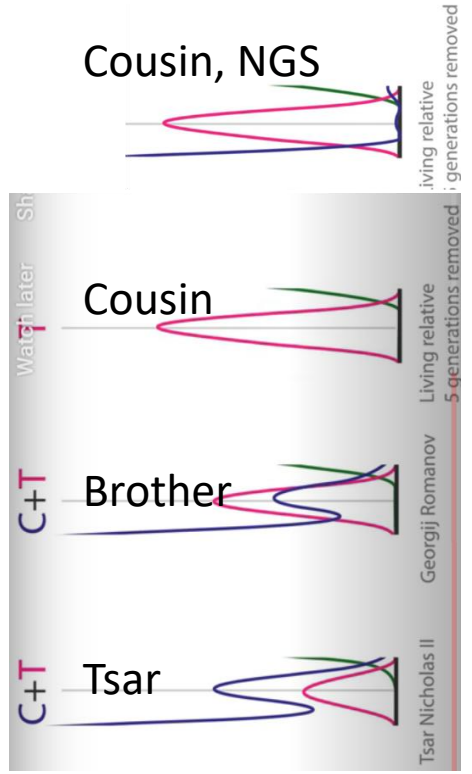
Princess Alice's mt-DNA (dau. of Queen Victoria) is the source for Children of the Tsar. Haplogroup H (263G, 315.1C, 16111T, 16357C)

How was the case solved?

Tested mitochondrial DNA, nucleic DNA and a Y chromosome marker

- Two common mt-DNA descendants, the Duke of Fife or Princess Xenia, matched the Tsar and his brother, Georgij. A difference at mt-DNA position 16,169 resulted in a heteroplasmy for the Tsar and his brother, Georgij, (C16169Y, 1:300K odds) which was lost in two of their known relatives, the Duke of Fife and Princess Xenia. Thus mt-DNA was able to prove that the mystery skeleton was indeed the murdered Tsar.
- Tsarina Alexandra of Russia and her children, Olga, Tatiana, Maria and Anastasia, were identified as belonging to mt-DNA Haplogroup H.
  - Anna Anderson who claimed that she was the missing Princess Anastasia was disproven to an heir.

Heteroplamic Position Sequence Data



Second Gravesite

Human remains of 2 burned skeletons exhumed from a grave discovered in July 2007, and the results of a comprehensive genomic analysis of remains from the first grave discovered in 1991.

Additionally, ≈117 years old archival blood specimens from Nicholas II were obtained and genotyped

Despite the severe damage to the bone specimens complete mt genome sequences and nuclear (especially Y chromosome) DNA were obtained.

The results of the studies provide unequivocal evidence that the remains of Nicholas II and his entire family, including all 5 children, have been identified.

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664067/

mt-DNA Analysis
Queen Victoria's mt DNA Type

This exact mt-DNA sequence pattern is not found in any other family (out of n=70K). Only 1 sample initially matched but further analysis showed that it had mismatches for position 524.1 and 524.2.

SNP position	263	315.1	524.1	524.2	750	1438	3010	4137	4769	8860	15326	16111	16357	16519
reference sequence	A	—	—	—	A	A	G	C	A	A	A	C	T	T
Alexi	G	C	A	C	G	G	A	T	G	G	G	T	C	C
Alexi†	G	C	A	C	G	G	A	T	G	G	G	T	C	C
Maria	G	C	A	C	G	G	A	T	G	G	G	T	C	C
Tsarina Alexandria	G	C	A	C	G	G	A	T	G	G	G	T	C	C
1. Victoria's Great grand dau.	G	C	A	C	G	G	A	T	G	G	G	T	C	C
2. Victoria's Great grand dau.	G	C	A	C	G	G	A	T	G	G	G	T	C	C
Frequency in Human Genome database	0.997	0.961	0.014	0.014	0.992	0.969	0.203	0.001	0.989	0.998	0.994	0.019	0.013	0.597

† Whole-genome amplification N= Reference and Romanov sequences differs.

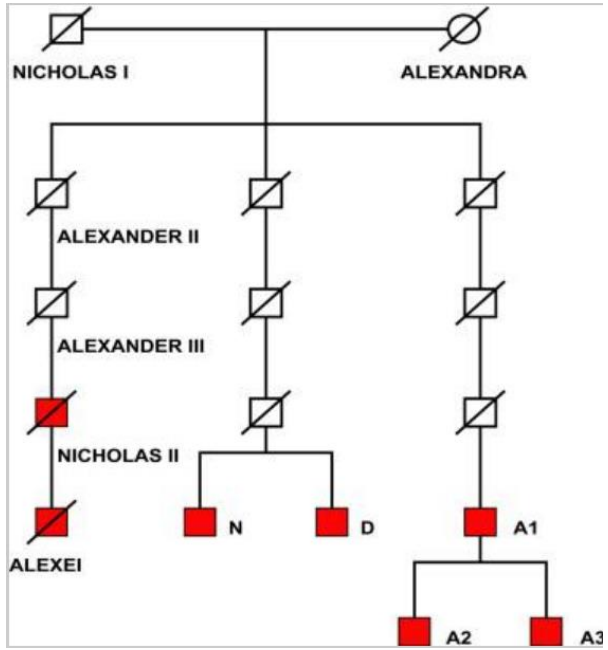
The frequencies of single nucleotide polymorphisms were obtained from mtDB-Human Mitochondrial Genome Database

The results of our studies provide unequivocal evidence that the remains of Nicholas II and his entire family, including all 5 children, have been identified.

In large population databases for HVR1 sequences, which include Russian, East and West European populations (Table S1), we found that this "Queen Victoria" mtDNA type (identical in putative remains of Empress Alexandra, her children and Queen Victoria's living descendants) is very rare in human populations. In a collection of >70,000 individuals with available HVR1 data we found only 1 individual with an identical profile

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664067/

Y-DNA Analysis



Marker:	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	Y-GATA-	DYS437	DYS438	DYS448
Nicholas II	16	13	24	29	17	14	11, 14	13	10	11	24	13	12	15	12	19
Alexi	16	13	24	29	17	14	11, nd	13	10	11	24	13	12	15	12	19
Archival Nicholas II bloodstain	16	13	24	29	17	14	11, 14	13	10	11	24	13	12	15	12	19
Romanov family members	16	13	24	29	17	14	11, 14	13	10	11	24	13	12	15	12	19
Control DNA ABI, 007	15	13	24	29	17	15	11, 14	13	11	12	24	13	13	15	12	19

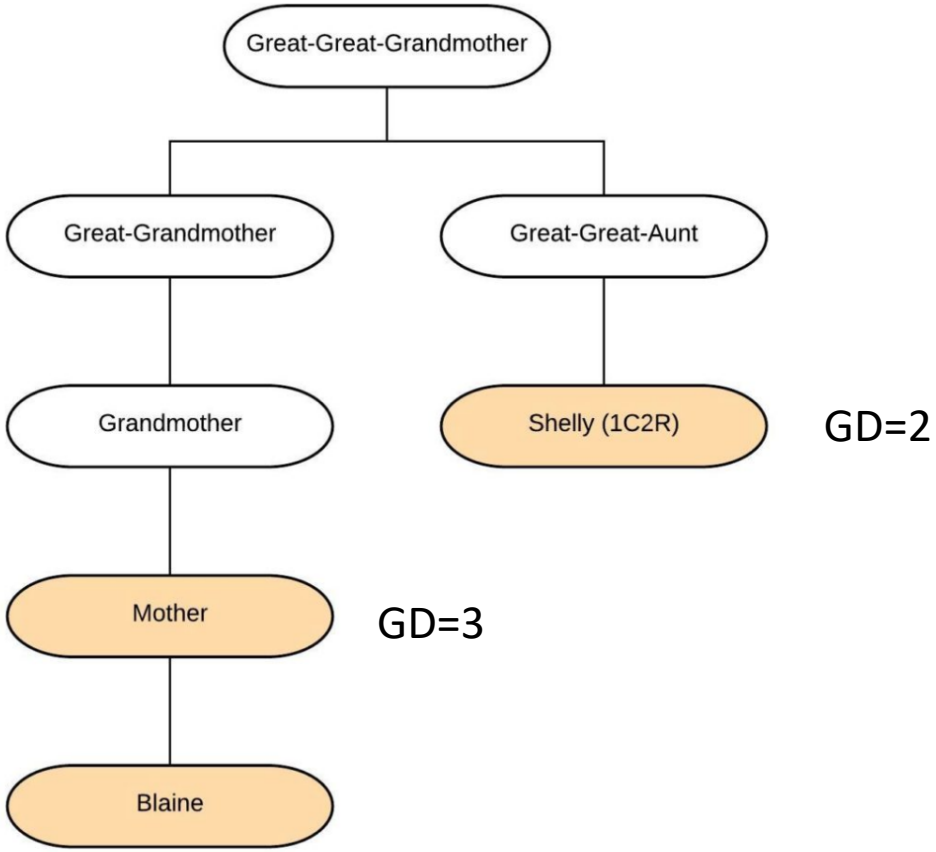
Y-DNA results also confirmed that Prince Alexi was found in the second grave site.

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664067/

Case Study 2: Blaine Bettinger's mt-DNA Enigma

- Blaine's mom had a GD value of 3 compared to him.
- Mitochondrial DNA gives a numerical value to how close your matches are to you.
- Usually, their closeness is in the order of: GD0 > GD1 > GD2 >GD3.
  - Note differences due to insertions/deletions at at 309 and 315 are not used for GD value.

Blaine Bettinger's mt-DNA Surprise



Blaine was closer to his great aunt's daughter than to his mother using Genetic Difference numbers. These values can be *misleading* especially when insertions/deletions in the poly C region are involved.

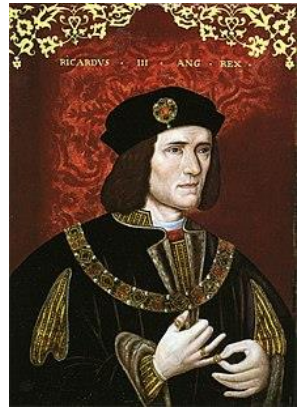
Blaine Bettinger's mt-DNA Mystery

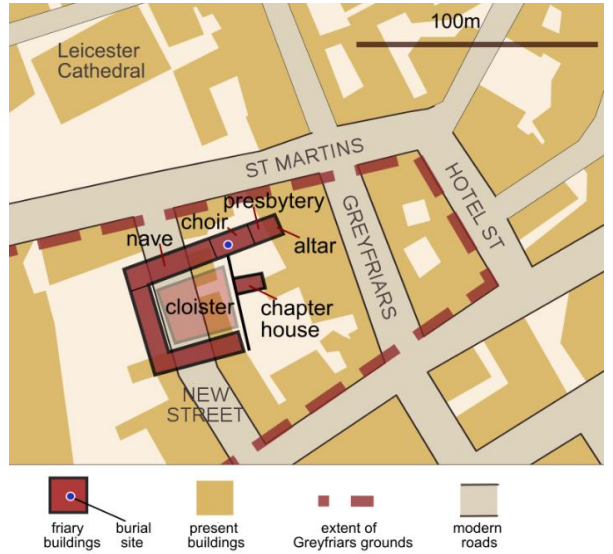
Tested	Difference from Reference Sequence	Position no./Sequence											
		567	568	569	570	571	572	573	573.1	573.2	573.3	573.4	16189
Grandson	573.1, 573.2	A	C	C	C	C	C	C	C	C	–	–	C
Daughter	16189Y, 573.1, 573.2, 573.3, 573.4	A	C	C	C	C	C	C	C	C	C	C	Y
1C2R	573.1, 573.2, 573.3, 573.5	A	C	C	C	C	C	C	C	C	C	C	C
		(–) = Absent. Y = C or T											

He had a second analysis done and found he was now a GD=2 from his mother. Heteroplasmy are reported only when the value is >20% of the total at a position.

Case Study 3: Power of Mitochondrial DNA testing
Identification of Remains of Richard III dead for >500 years

- Richard III of England (1452 - 1485) was killed fighting the forces of Henry Tudor at the Battle of Bosworth in 1485, the last major battle of the Wars of the Roses. His burial place was reported as being at Greyfriars convent of Franciscan monks in Leicester.
- Greyfriars Cloister was dissolved/demolished in 1538 by Henry VIII and the location of Richard's tomb lost to time.
- In 2012 a grave with an almost intact skeleton was uncovered during an archaeological study of the former cloister resulting in three trenches being dug across the parking area behind the buildings on Greyfriars.
- Richard III left no living descendants.





Source: https://le.ac.uk/richard-iii

Richard III of England (1452 - 1485)

- Historical information about various features of his life and death exists. These include aspects of his physical appearance such as having a slim build, one shoulder higher than the other and that he suffered battle injuries, which resulted in his death.
- The archaeological, osteological and radiocarbon dating evidence were all consistent with the remains being those of Richard III:
  - The skeleton was that of a male aged 30 to 34 years, with severe scoliosis rendering one shoulder higher than the other, and numerous perimortem battle injuries (11 injuries).
  - Radiocarbon dating gave a date range of 1456–1530 AD at a 95.4% probability.
- These match his physical features and are consistent with his date of death.
- What was missing was genetic and genealogical data.

Richard III of England (1452 - 1485)

- “The family trees of noble families and other landed elites are often better recorded and a family tree showing an unbroken female lineage tracing from Anne of York, Richard’s eldest sister, down to the early 19th century” and a modern descendant family identified.
- The researchers carried out additional genealogical research to fully document this first lineage and, furthermore, traced a second female lineage.
- The Y-chromosome haplotype from the skeleton did not match that of male-line relatives of Richard III, but this is not remarkable given that a false-paternity event could have occurred in any of the intervening generations.

Power of Mitochondrial DNA testing

Identification of the Remains of Richard III dead for >500 years

After several generations, only the uniparentally inherited **mitochondrial** genome and nonrecombining part of the **Y-chromosome** can be informative about relatedness. So, it's perfect for testing relatedness over many generations.

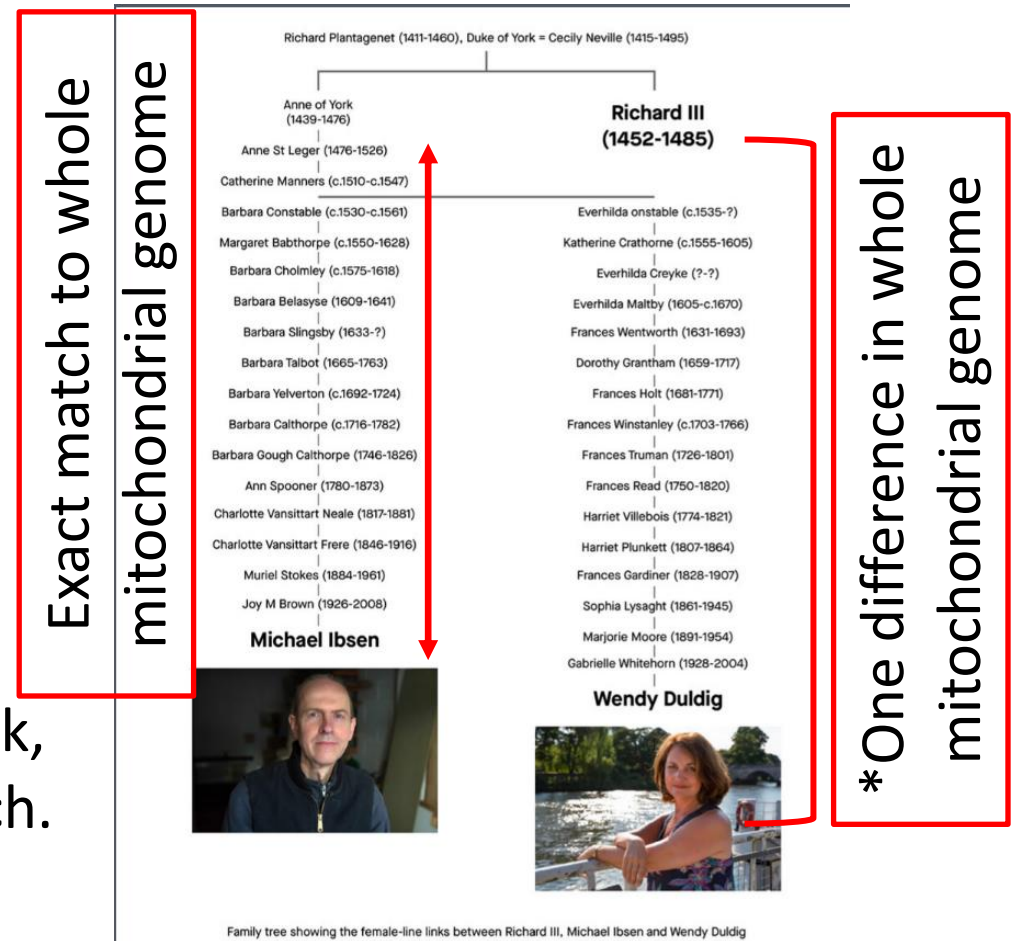
- 5 male descendants were tested, none matched Richard III's Y-haplotype, G-P287.

  mt-DNA match

- Two female line descendants of Anne of York, Richard's eldest sister were tested and match.

Source: https://www.nature.com/articles/ncomms6631

Richard III (1452 – 1485)
17 degrees | 19 degrees

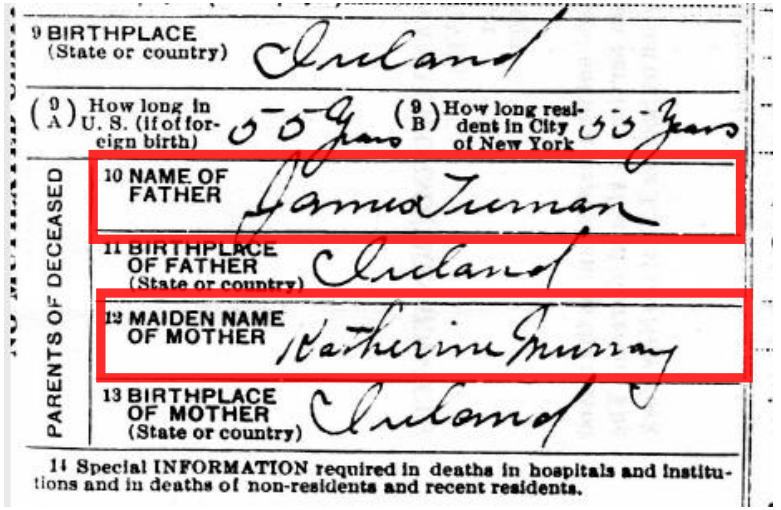


Family tree showing the female-line links between Richard III, Michael Ibsen and Wendy Duldig

*One difference at position 8994, G8994A
All three had 315.1 C insertion

Case Study 4: What town in Ireland was Ellen Tiernan from and who were her parents / grandparents?

- Ellen's parents mentioned on her death certificate and indicated that Ellen was born in Ireland to James Tiernan and Katherine Murray.
- No town reported.


9 BIRTHPLACE (State or country) Ireland

(9 A) How long in U. S. (if of foreign birth) 0-0-9 Years (9 B) How long resident in City of New York 55 Years

PARENTS OF DECEASED

10 NAME OF FATHER James Tiernan

11 BIRTHPLACE OF FATHER (State or country) Ireland

12 MAIDEN NAME OF MOTHER Katherine Murray

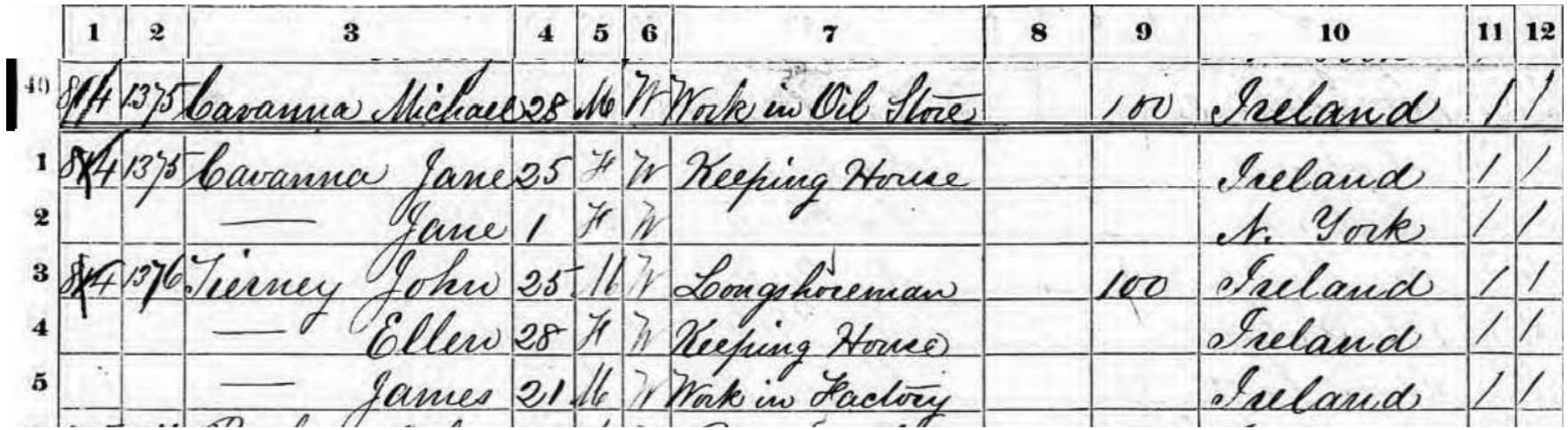
13 BIRTHPLACE OF MOTHER (State or country) Ireland

14 Special INFORMATION required in deaths in hospitals and institutions and in deaths of non-residents and recent residents.

1914 NYC Death Certificate
of Ellen Tiernan
my 1st GGM

US Census Records List Ellen's brothers

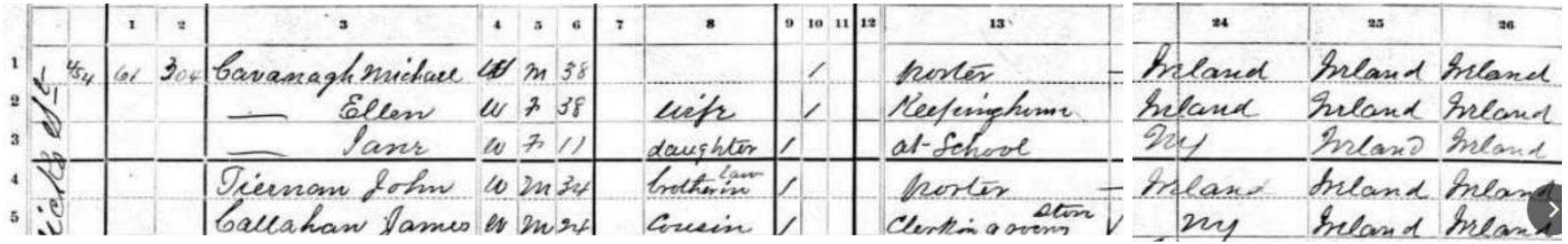
1870 United States Federal Census for Cavanagh/Tiernan, New York, Kings, Brooklyn Ward 06, Pages 177-8



	1	2	3	4	5	6	7	8	9	10	11	12
40	8/4	1375	Cavanna Michael	28	M	W	Work in Oil Store		100	Ireland	/	/
1	8/4	1375	Cavanna Jane	25	F	W	Keeping House			Ireland	/	/
2			— Jane	1	F	W				N. York	/	/
3	8/4	1376	Tierney John	25	M	W	Longshoreman		100	Ireland	/	/
4			— Ellen	28	F	W	Keeping House			Ireland	/	/
5			— James	21	M	W	Work in Factory			Ireland	/	/

Ellen's brother

1880 United States Federal Census for Cavanagh household, New York, Kings, Brooklyn, ED: 5, Page 30



		1	2	3	4	5	6	7	8	9	10	11	12	13	24	25	26
1	454	61	304	Cavanagh Michael	W	M	38				/			porter	Ireland	Ireland	Ireland
2				— Ellen	W	F	38		wife		/			Keepinghome	Ireland	Ireland	Ireland
3				— Jane	W	F	11		daughter	/				at-School	Ire	Ireland	Ireland
4				Tiernon John	W	M	34		brotherin Law	/				porter	Ireland	Ireland	Ireland
5				Callahan James	W	M	24		Cousin	/				Clerk in a store	Ire	Ireland	Ireland

In 1870 census is Michael's wife Jane(⬅)? Is Jane's mother Jane in 1870, if so, is Ellen(⬅) her step-mother in 1880? Unlike the 1880 census, the 1880 census lists relationships and reveals Ellen is probably also the wife in 1870.

Finding Where in Ireland Ellen Tiernan's Family and Maternal Ancestors Originated

- Looked for Ellen Tiernan or Tierney born ~1842 in Irish birth records. There were 11 candidates between 1833 and 1844 but none found with the same parents named on her death certificate.
- Next, searched in Ireland for other family members by looking for her two brothers, John and James, with the same birth year found on the census and having the same parents.

Searched for her brothers at RootsIreland.ie

Search:	John Tiernan born ~1846						
	Source	**Surname**	**First Name**	**Year**	**County**		
	Church Baptism	Tiernan	John	1850	Co. Louth		
	Church Baptism	Teirney	John	1841	Co. Tyrone		
	Church Baptism	MacTernan	John	1842	Co. Leitrim		
	Church Baptism	Tiernan	John	1845	Co. Roscommon	match Ellen's brother's birth year	no match with parents
Search:	James Tiernan born ~1849						
	Source	**Surname**	**First Name**	**Year**	**County**		
	Church Baptism	Tiernan	James	1849	Co. Roscommon	match Ellen's brother's birth year	matches parents
	Church Baptism	Tiernan	James	1851	Co. Roscommon		
	Church Baptism	Tiernan	James	1852	Co. Mayo		
	Church Baptism	MacTernan	James	1853	Co. Leitrim		
	Church Baptism	MacTeirnan	James	1846	Co. Leitrim		
	Church Baptism	Tiernan	James	1847	Co. Dublin		
	Church Baptism	Teirney	James	1848	Co. Meath		
	Church Baptism	Tiernan	James	1848	Co. Louth		

Church Baptism Record

Name:	James Tiernan	Date of Baptism/Birth:	06-Sep-1849
Address:	Cartron	Parish/District:	ELPHIN
Gender:	Male	County	Co. Roscommon
		Denomination:	Roman Catholic
	James Tiernan		
Father:	*	Mother:	Catherine Murry
Occupation:			
Sponsor 1 / Informant 1:	Thomas Kelly	Sponsor 2 / Informant 2:	Brigid Tiernan
Note:	Fr Noonan		

1. A James Tiernan match found with same parents
2. No baptism record found for John with same parents as James and Ellen.

Found two males but only one with the same parents as Ellen and his birth locations was Tullycartron, County Roscommon (near the town of Elphin). Interestingly, Ellen's husband Michael was also from Elphin.

Evidence for Catherine Murray as My 3rd GGM and Catharine Garaghty as my 4th GGM

Report from County Roscommon, Ireland professional genealogist on the James & Catherine Tiernan's Family from Elphin Parish.

Baptismal records of the family of James Tiernan and Catherine Murray

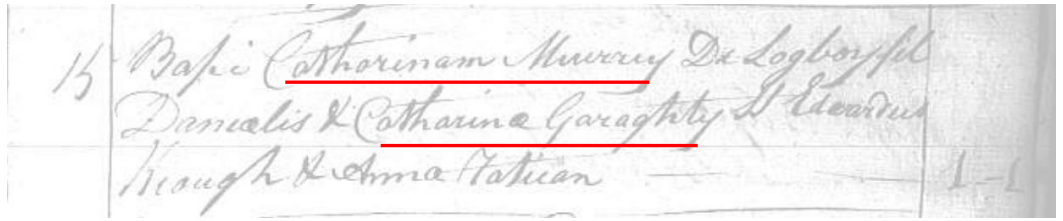
NAME	Bridget Ternan
TOWNLAND ADDRESS	Not recorded
SOURCE	Elphin Roman Catholic Parish Records
PARENTS	James Ternan and Catherine Murry
DATE OF BIRTH	Not recorded
DATE OF BAPTISM	27th January 1833
BAPTISMAL SPONSORS	Michael Murry and Grace McDermott

NAME	Mary Tiernan
TOWNLAND ADDRESS	Not recorded
SOURCE	Elphin Roman Catholic Parish Records
PARENTS	James Tiernan and Catherine Murry
DATE OF BIRTH	Not recorded
DATE OF BAPTISM	17th February 1837
BAPTISMAL SPONSORS	Patrick Toolan and Bridget Murry

NAME	Catherine Tiernan
TOWNLAND ADDRESS	Tully
SOURCE	Elphin Roman Catholic Parish Records
PARENTS	James Tiernan and Catherine Murry
DATE OF BIRTH	Not recorded
DATE OF BAPTISM	29th April 1839
BAPTISMAL SPONSORS	Michael Casserly and Margaret Casserly

NAME	Michael Tiernan
TOWNLAND ADDRESS	Tully
SOURCE	Elphin Roman Catholic Parish Records
PARENTS	James Tiernan and Catherine Murray
DATE OF BIRTH	Not recorded
DATE OF BAPTISM	2nd November 1843
BAPTISMAL SPONSORS	Michael Neary and Biddy Neary

Catherine Murray shown by red arrow (⬅)



The 1810 baptism record for Catherine Murray Elphin RC Parish, Co. Roscommon indicates her mother was Catherine Garaghty.

My Maternal Mitochondrial Line

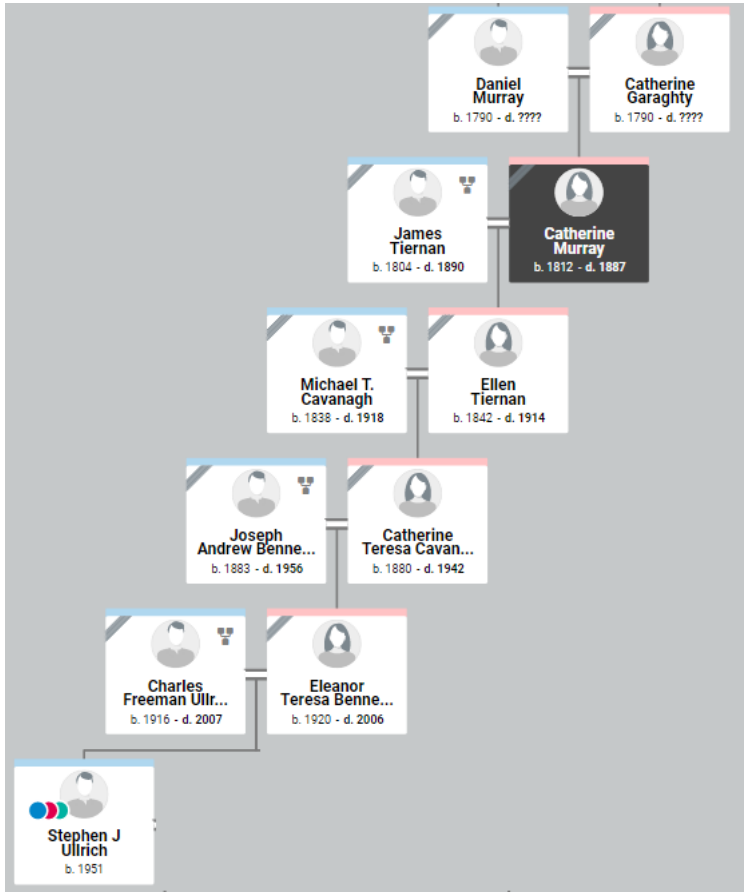
WikiTree tool

Mitochondrial DNA

Mitochondrial DNA is passed down from mother to child. Here are Stephen's direct maternal line ancestors.

Eleanor Teresa Bennett (15 Apr 1920 - 16 Apr 2006) DNA
Catherine Teresa Cavanagh (28 Nov 1880 - 25 May 1942) [confident]
Ellen Tiernan (14 Apr 1842 - 18 Nov 1914) [confident]
Catherine Murray (1812 - 13 Feb 1887) [confident]
Catherine Garaghty (abt 1788 -)
[Fourth Great-Grandmother Unknown]

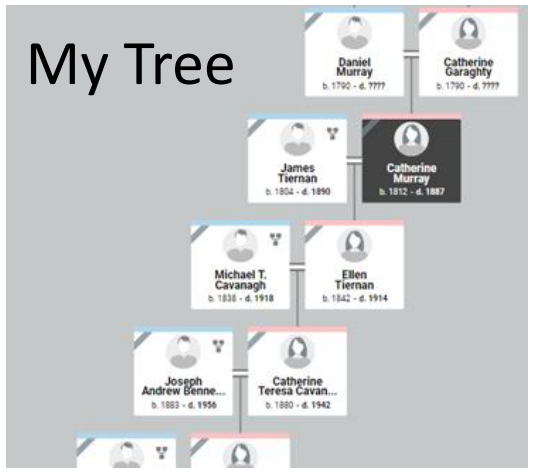
Proof for oldest maternal line ancestor: sharing mitochondrial DNA with another descendant

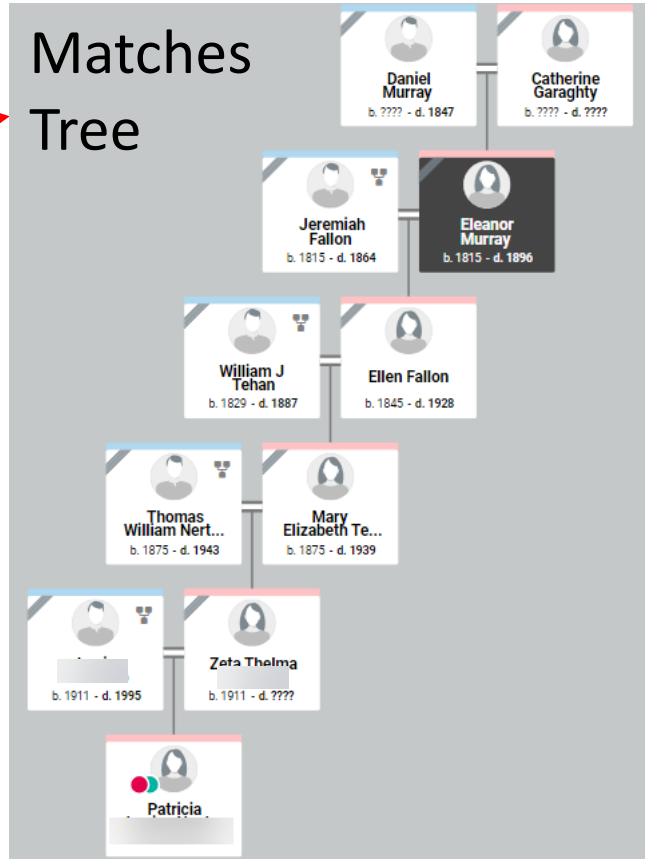


My Maternal Mt DNA Match

HVR1, HVR2, CODING REGIONS - 4 MATCHES

Genetic Distance	Name	Earliest Known Ancestor	mtDNA Haplogroup	Match Date
0	Patricia [redacted] FMS FF	Tehan,Fallon,Murray,Garaghty	HV1c	7/29/2022
0	Marjorie [redacted] FMS		HV1c	7/29/2022





People in black background are sisters

Proof of oldest maternal line ancestor: Sharing mt-DNA with another descendant

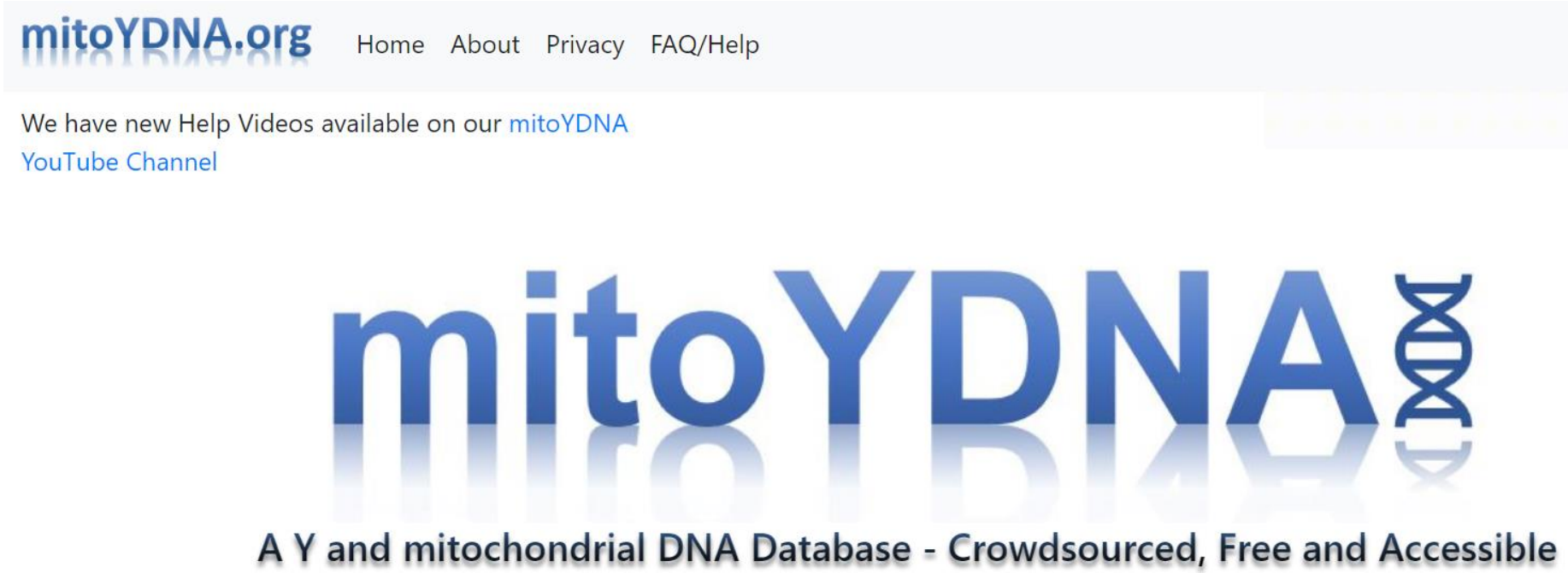
Million Mito Project – FT DNA
Growing the Family Tree of Womankind

- Million Mito Project's aim is for 1 million mt-DNA tests. Currently (Oct 2023), there are 214,000 mitochondrial tests in the database. (Will use samples from Family Tree DNA, the Genographic Project, and academic tests).
- Develop automated software to handle very large numbers of mt-DNA sequences must be adapted or developed.
- Highly refined haplogroups will improve the ability to use mitochondrial DNA for genealogical purposes – similar to what the Big Y-700 SNP testing and the expanded Y haplotree has done for Y DNA analysis.

https://dna-explained.com/2022/04/13/million-mito-project-team-introduction-and-progress-update/

mitoYDNA.org

A site where you can upload your mitochondrial and Y DNA haplogroups and find matches



URL: https://www.mitoydna.org/

Further Resources and Reading

- Mito DNA Haplotree: https://www.familytreedna.com/public/mt-dna-haplotree/L
- Family History Fanatics, Maternal Haplogroups: https://www.youtube.com/watch?v=09GsPp-iIJM
- RootsTech presentation Dr. Paul Meier's **Review of the Million Mito Project**: https://www.youtube.com/watch?v=cpctoeKb0Kw (available as of ~Oct. 2023)
- Roberta Estes, DNAExplained, Mito DNA blogs: https://dna-explained.com/mitochondrial-dna/
- FamilyTree Webinar Roberta Estes - **Wringing Every Drop out of Mitochondrial DNA**
- ($) https://familytreewebinars.com/webinar/wringing-every-drop-out-of-mitochondrial-dna/

Thank You