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INTRODUCING MPS TO A FORENSIC WORKFLOW – WHERE TO START?

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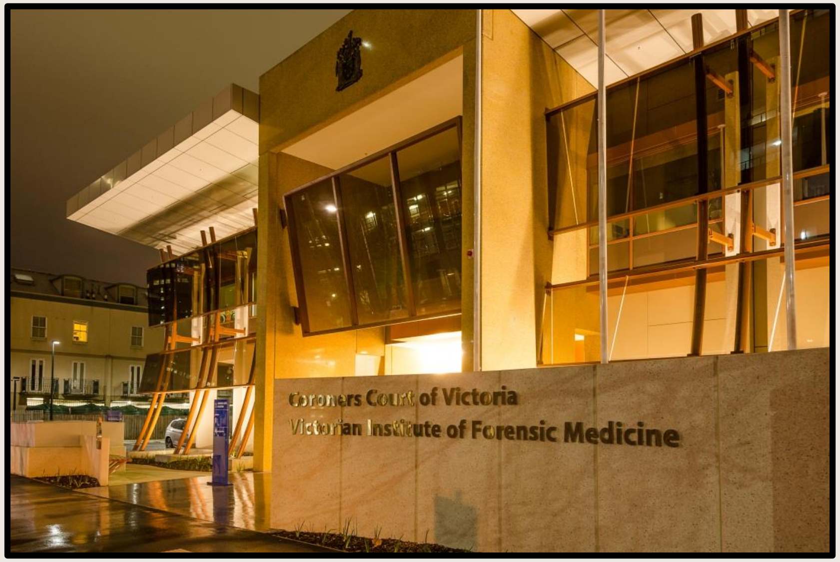
Warning: contains images of deceased persons

HIDS, *June 2019*

VICTORIA - AUSTRALIA

- **Coroners Court of Victoria (CCoV) is required to investigate any reportable death**
    - Annually, over 6,500 cases are admitted as coronial cases
- **Victorian Institute of Forensic Medicine (VIFM)**
    - Independent forensic medical services to the Victorian public and justice system
- **Identification must be established to enable the legal interment of the deceased**
    - Most cases are identifiable using visual inspections
    - ~ 10 % of reportable cases required ID by scientific means
        - DNA
        - Fingerprints
        - Dental



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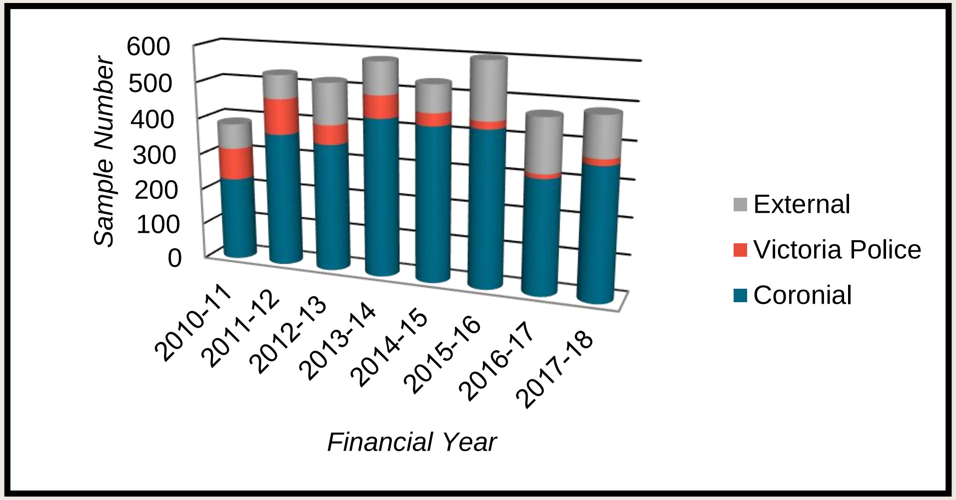
MOLECULAR BIOLOGY LABORATORY (MBL)

- **Coronial identification**
  - Nuclear DNA (nDNA) – 24 markers (including sex determination markers)
  - Mitochondrial DNA (mtDNA)
- **Disaster Victim Identification (DVI)**
  - Two or more persons killed in an event
  - Multiple fatality car crash to 2009 Victorian Bushfires
- **Missing persons investigations**
  - Unidentified remains reconciled with missing persons
- **DNA testing services**
  - mtDNA analysis
  - ID verification



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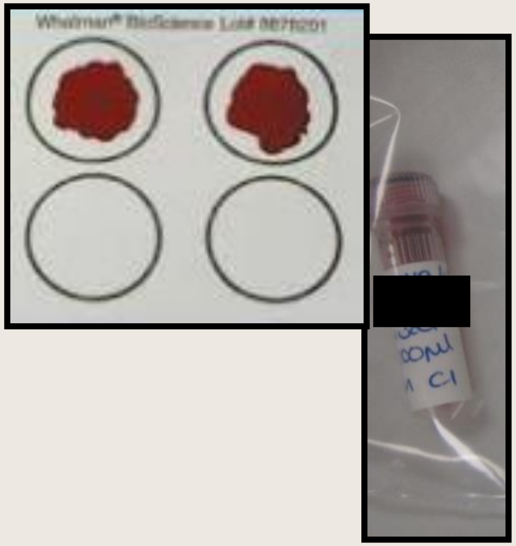
DNA ANALYSIS PIPELINE

- **PM samples**
  - **Blood**: liquid; stain collected on FTA card or swab
  - **Tissue**: deep red muscle tissue (~ 1g)
  - **Toenails**: few toe nail clippings or whole toenail
  - **Bone**: compact bone – preferably head of femur
  - **Bladder swabs**: cells lining the bladder wall are collected on a swab



[redacted]

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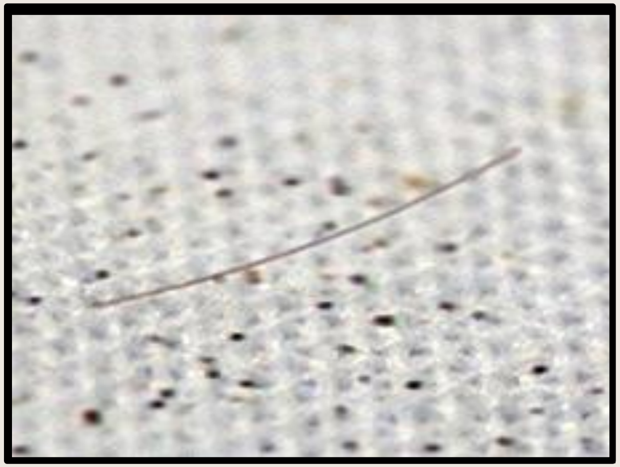
DNA ANALYSIS PIPELINE

- ❑ **Other samples**
  - **Fixed tissue**: paraffin embedded or slides
  - **Urine**: fresh or frozen
  - **Hair**: with or without the hair root

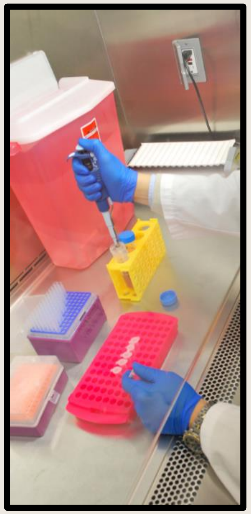


WORKFLOW

DNA Extraction

DNA investigator kit

Organic



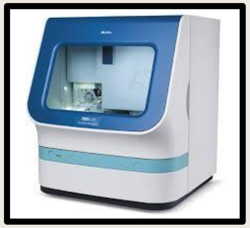
DNA Quant

Quantifiler™ HP kit

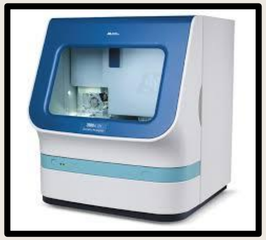
DNA 1000

DNA Profiling

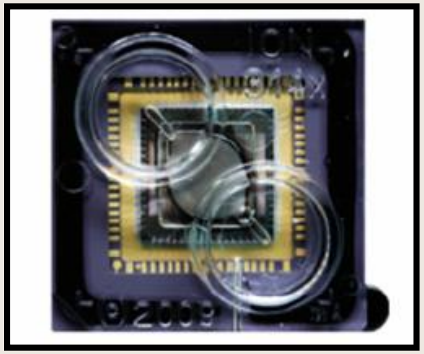
GlobalFiler™ and Minifiler™ kits



BigDye™ Terminator Cycle Sequencing Kits

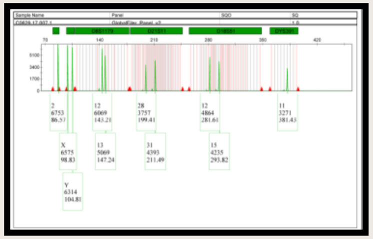


Massive Parallel Sequencing (MPS)

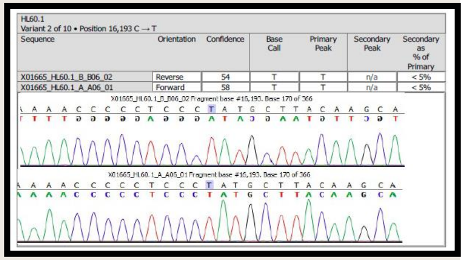


DNA Analysis

GeneMapper™ ID-X Software



Sequencher Software



HL60.1
Variant 2 of 10 • Position 16,193 C → T

Sequence	Orientation	Confidence	Base Call	Primary Peak	Secondary Peak	Secondary as % of Primary
X01665_HL60.1_B_B06_02	Reverse	54	T	T	n/a	< 5%
X01665_HL60.1_A_A06_01	Forward	58	T	T	n/a	< 5%

X01665_HL60.1_B_B06_02 Fragment base #16,193. Base 170 of 366

X01665_HL60.1_A_A06_01 Fragment base #16,193. Base 170 of 366

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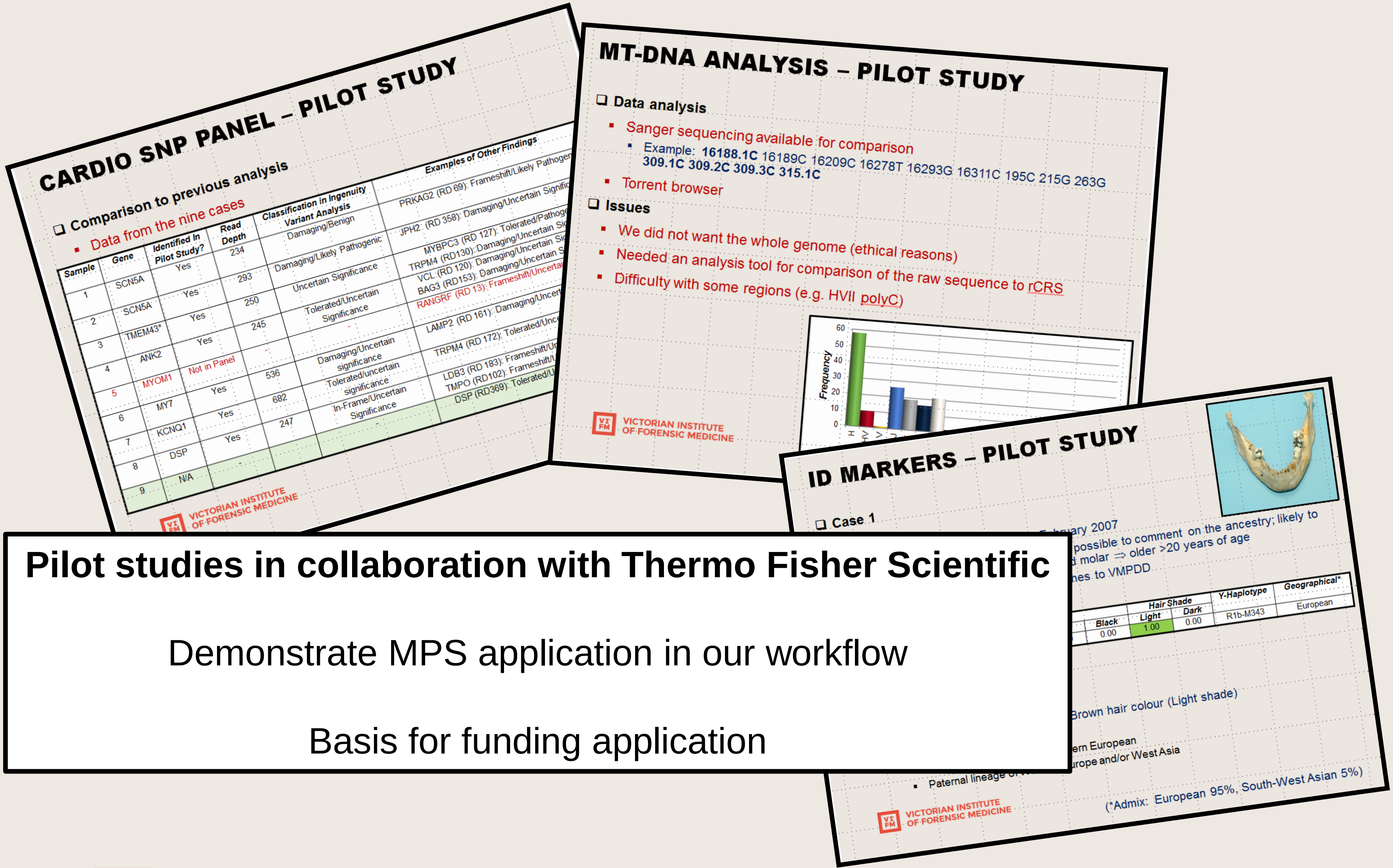
MASSIVE PARALLEL SEQUENCING (MPS)



CARDIO SNP PANEL – PILOT STUDY

- ☐ Comparison to previous analysis
  - Data from the nine cases

Sample	Gene	Identified In Pilot Study?	Read Depth	Classification in Ingenuity Variant Analysis	Examples of Other Findings
1	SCN5A	Yes	234	Damaging/Benign	PRKAG2 (RD 69): Frameshift/Likely Pathogen
2	SCN5A	Yes	293	Damaging/Likely Pathogenic	JPH2 (RD 358): Damaging/Uncertain Signific
3	TMEM43*	Yes	250	Uncertain Significance	MYBPC3 (RD 127): Tolerated/Pathoge TRPM4 (RD130): Damaging/Uncertain Sig VCL (RD 120): Damaging/Uncertain Sig BAG3 (RD153): Damaging/Uncertain S
4	ANK2	Yes	245	Tolerated/Uncertain Significance	RANGRF (RD 13): Frameshift/Uncertai
5	MYOM1	Not in Panel	-	-	LAMP2 (RD 161): Damaging/Uncert
6	MY7	Yes	536	Damaging/Uncertain significance	TRPM4 (RD 172): Tolerated/Unce
7	KCNQ1	Yes	682	Tolerated/uncertain significance	LDB3 (RD 183): Frameshift/Un TMPO (RD102): Frameshift/U
8	DSP	Yes	247	In-Frame/Uncertain Significance	DSP (RD369): Tolerated/U
9	N/A	-		-	

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MT-DNA ANALYSIS – PILOT STUDY

- ☐ Data analysis
  - Sanger sequencing available for comparison
    - Example: **16188.1C** 16189C 16209C 16278T 16293G 16311C 195C 215G 263G **309.1C 309.2C 309.3C 315.1C**
  - Torrent browser
- ☐ Issues
  - We did not want the whole genome (ethical reasons)
  - Needed an analysis tool for comparison of the raw sequence to rCRS
  - Difficulty with some regions (e.g. HVII polyC)

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ID MARKERS – PILOT STUDY

- ☐ Case 1

bruary 2007

possible to comment on the ancestry; likely to

d molar ⇒ older >20 years of age

hes to VMPDD

	Black	Hair Shade Light	Hair Shade Dark	Y-Haplotype	Geographical*
	0.00	1.00	0.00	R1b-M343	European

Brown hair colour (Light shade)

ern European

urope and/or West Asia

- Paternal lineage o

(*Admix: European 95%, South-West Asian 5%)

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Pilot studies in collaboration with Thermo Fisher Scientific

Demonstrate MPS application in our workflow

Basis for funding application

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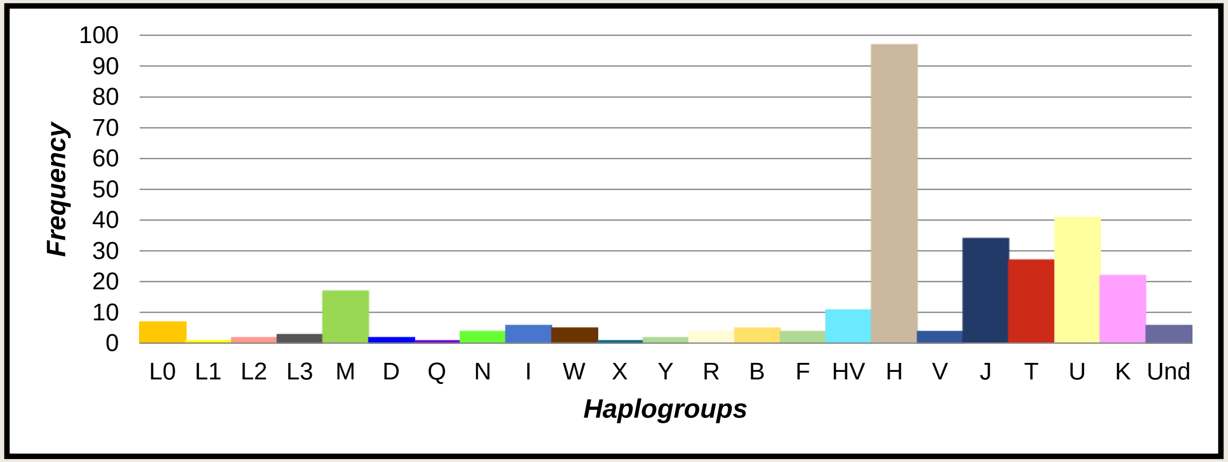
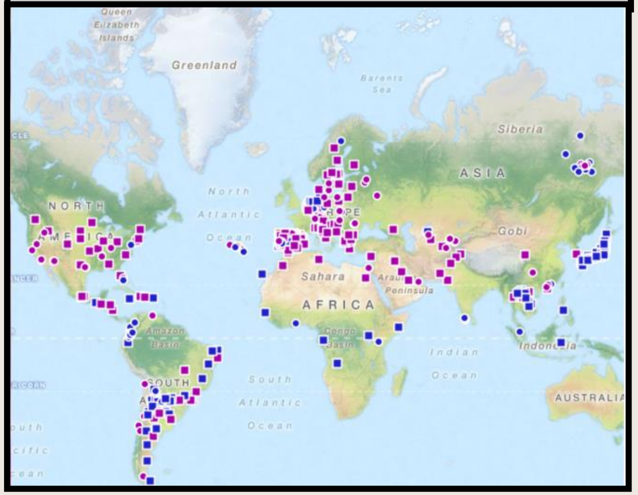
MT-DNA ANALYSIS – PILOT STUDY

- ❑ **Victorian mtDNA haplotype database project**
  - Frequency of the reported mtDNA profile (haplotype) in the population
  - Use EMPOP (EDNAP mtDNA Population Database) - database did not contain Australian haplotype information
  - mtDNA analysis of ~ 300 volunteer samples
- ❑ **Early Access HID-Ion AmpliSeq Mitochondrial Tiling Panel**
  - 10 samples at full and half AmpliSeq reactions
  - 58 samples analysed in total
  - Using Ion Chef to generate templates
  - Sequenced on 318v2 chips

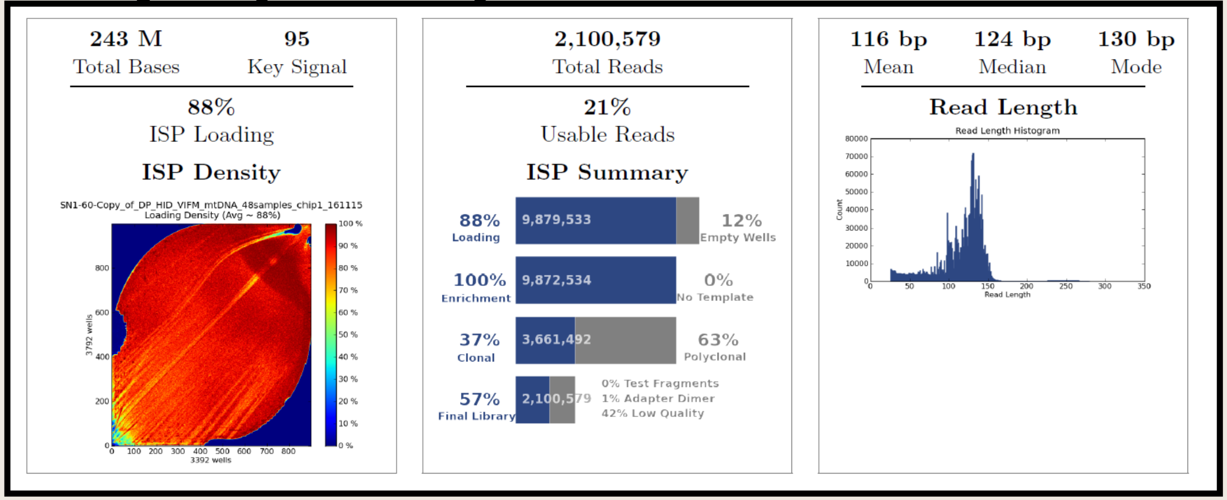


mtDNA database, v4/R12



MT-DNA ANALYSIS – PILOT STUDY

❑ Quality of library construction



243 M Total Bases

95 Key Signal

88% ISP Loading

ISP Density

2,100,579 Total Reads

21% Usable Reads

ISP Summary

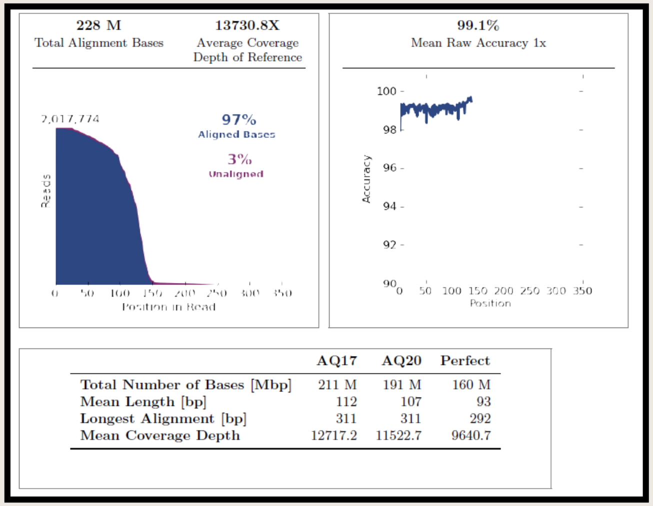
88% Loading	9,879,533	12% Empty Wells
100% Enrichment	9,872,534	0% No Template
37% Clonal	3,661,492	63% Polyclonal
57% Final Library	2,100,579	0% Test Fragments 1% Adapter Dimer 42% Low Quality

116 bp Mean

124 bp Median

130 bp Mode

Read Length



228 M Total Alignment Bases

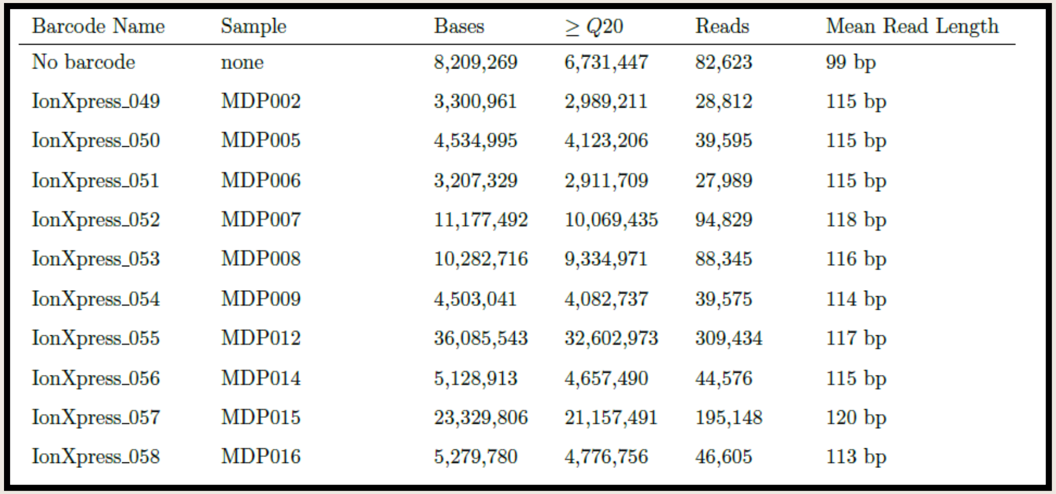
13730.8X Average Coverage Depth of Reference

99.1% Mean Raw Accuracy 1x

	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	211 M	191 M	160 M
Mean Length [bp]	112	107	93
Longest Alignment [bp]	311	311	292
Mean Coverage Depth	12717.2	11522.7	9640.7

❑ Bioinformatics

- Ion Torrent – Ion Reporter
- Sequencher 5.4
- IGV 2.3



Barcode Name	Sample	Bases	$\geq Q20$	Reads	Mean Read Length
No barcode	none	8,209,269	6,731,447	82,623	99 bp
IonXpress_049	MDP002	3,300,961	2,989,211	28,812	115 bp
IonXpress_050	MDP005	4,534,995	4,123,206	39,595	115 bp
IonXpress_051	MDP006	3,207,329	2,911,709	27,989	115 bp
IonXpress_052	MDP007	11,177,492	10,069,435	94,829	118 bp
IonXpress_053	MDP008	10,282,716	9,334,971	88,345	116 bp
IonXpress_054	MDP009	4,503,041	4,082,737	39,575	114 bp
IonXpress_055	MDP012	36,085,543	32,602,973	309,434	117 bp
IonXpress_056	MDP014	5,128,913	4,657,490	44,576	115 bp
IonXpress_057	MDP015	23,329,806	21,157,491	195,148	120 bp
IonXpress_058	MDP016	5,279,780	4,776,756	46,605	113 bp

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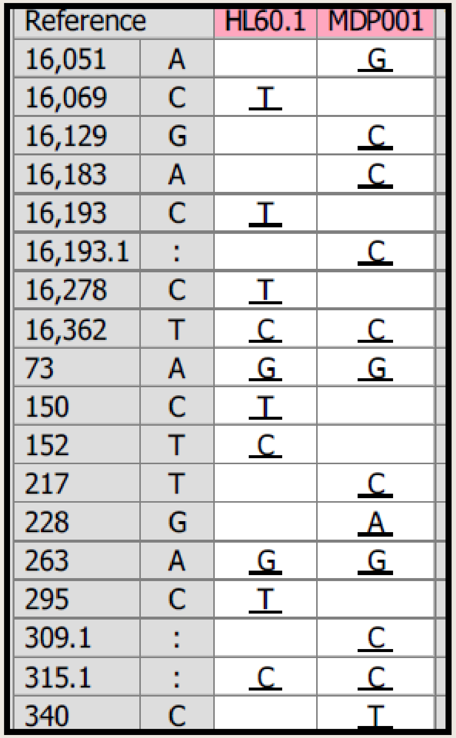
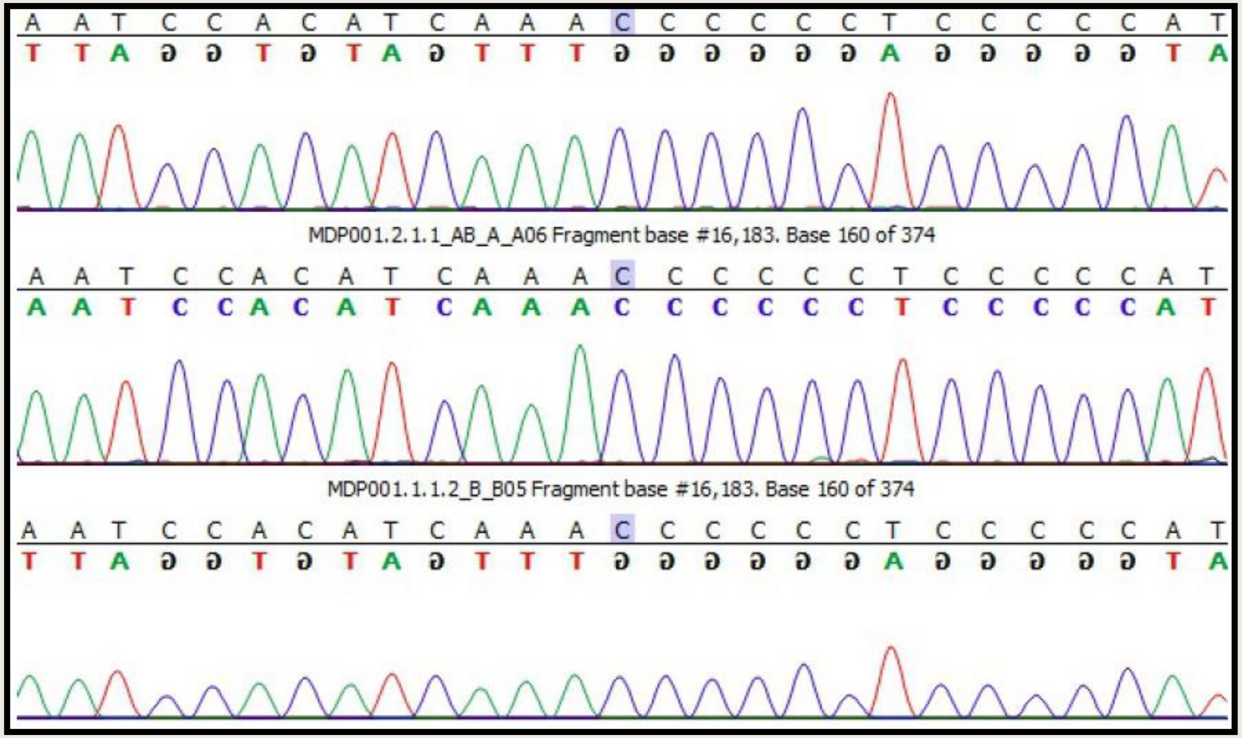
MT-DNA ANALYSIS – PILOT STUDY

❑ Example MDP001

- Sanger sequencing available for comparison
  - HVI (16024-16404) 16051G 16129C 16183C **16193.1C** 16362C
  - HVII (18-413) 73G 217C 228A 263G **309.1C** **315.1C** 340T



Reference		HL60.1	MDP001
16,051	A		G
16,069	C	T	
16,129	G		C
16,183	A		C
16,193	C	T	
16,193.1	:		C
16,278	C	T	
16,362	T	C	C
73	A	G	G
150	C	T	
152	T	C	
217	T		C
228	G		A
263	A	G	G
295	C	T	
309.1	:		C
315.1	:	C	C
340	C		T



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MT-DNA ANALYSIS – PILOT STUDY

❑ Example MDP001

- Torrent browser

HVI (16024-16404) **16051G** 16129C 16183C **16193.1C** 16362C
HVII (18-413) 73G 217C 228A 263G **309.1C** **315.1C** 340T

HALF REACTION

Chrom	Position	Ref	Variant	Allele Call	Filter	Frequency	Quality	Filter	Type	Allele Source	Allele Name	Gene ID	Region Name
chrM	73	A	G	Homozygous	-	100	7601.75	-	SNP	Novel	---	unknown	mt_1
chrM	217	T	C	Homozygous	-	100	18465.5	-	SNP	Novel	---	unknown	mt_2
chrM	228	G	A	Homozygous	-	100	18337.9	-	SNP	Novel	---	unknown	mt_2
chrM	263	A	G	Homozygous	-	100	2210.39	-	SNP	Novel	---	unknown	mt_3
chrM	311	-	C	Homozygous	-	100	2213.88	-	INS	Novel	---	unknown	mt_4
chrM	340	C	T	Homozygous	-	100	2184.29	-	SNP	Novel	---	unknown	mt_4
chrM	16051	A	G	Homozygous	-	96.8	14691.1	-	SNP	Novel	---	unknown	mt_156
chrM	16052	C	G	Heterozygous	-	20.7	316.78	-	SNP	Novel	---	unknown	mt_156
chrM	16129	G	C	Homozygous	-	100	13476.7	-	SNP	Novel	---	unknown	mt_158
chrM	16183	A	C	Heterozygous	-	87.6	1848.3	-	SNP	Novel	---	unknown	mt_158
chrM	16190	-	C	Homozygous	-	98.1	2224.15	-	INS	Novel	---	unknown	mt_158
chrM	16362	T	C	Homozygous	-	100	13455.5	-	SNP	Novel	---	unknown	mt_160

FULL REACTION

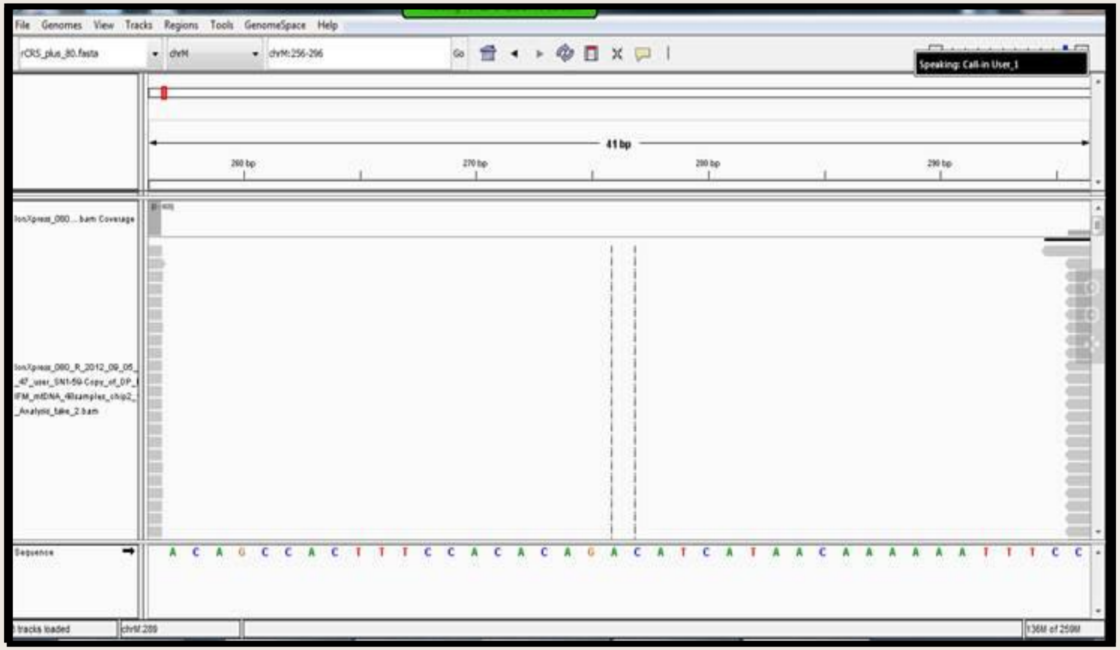
Chrom	Position	Ref	Variant	Allele Call	Filter	Frequency	Quality	Filter	Type	Allele Source	Allele Name	Gene ID	Region Name
chrM	73	A	G	Homozygous	-	100	32137	-	SNP	Novel	---	unknown	mt_1
chrM	217	T	C	Homozygous	-	100	19586.9	-	SNP	Novel	---	unknown	mt_2
chrM	228	G	A	Homozygous	-	100	19719	-	SNP	Novel	---	unknown	mt_2
chrM	263	A	G	Homozygous	-	100	7749.24	-	SNP	Novel	---	unknown	mt_3
chrM	311	-	C	Homozygous	-	100	5787.6	-	INS	Novel	---	unknown	mt_4
chrM	340	C	T	Homozygous	-	100	3770.41	-	SNP	Novel	---	unknown	mt_4
chrM	16051	A	G	Homozygous	-	100	21164.2	-	SNP	Novel	---	unknown	mt_156
chrM	16052	C	G	Heterozygous	-	26.1	953.52	-	SNP	Novel	---	unknown	mt_156
chrM	16129	G	C	Homozygous	-	100	38364.7	-	SNP	Novel	---	unknown	mt_158
chrM	16183	A	C	Homozygous	-	93.2	4572.08	-	SNP	Novel	---	unknown	mt_158
chrM	16190	-	C	Homozygous	-	100	5002.64	-	INS	Novel	---	unknown	mt_158
chrM	16362	T	C	Homozygous	-	100	26152.8	-	SNP	Novel	---	unknown	mt_160

MT-DNA ANALYSIS – PILOT STUDY

- ☐ **Comparison: non-concordance**
  - 58 of 58 samples incorrect calling of cytosine homopolymeric regions in HVI and HVII
  - Non-SWGDAM nomenclature used by Ion Reporter
    - i.e. Sample 49 variants "16187T 16188G 16189C" reported as "16187 TGC"
  - 1 missed insertion 291.1(non C repeat region)
  - 3 missed deletions at 249
  - ~~2 samples with missed variants at 150 and 152~~
  - ~~2 samples with missed variant at 150 only~~
  - 1 sample with missed variants at 263, 295
  - ~~1 sample with missed variant at 16296~~
- ☐ **Amendment to Torrent Variant Caller Analysis parameter settings**
  - realignment-threshold = 0.9

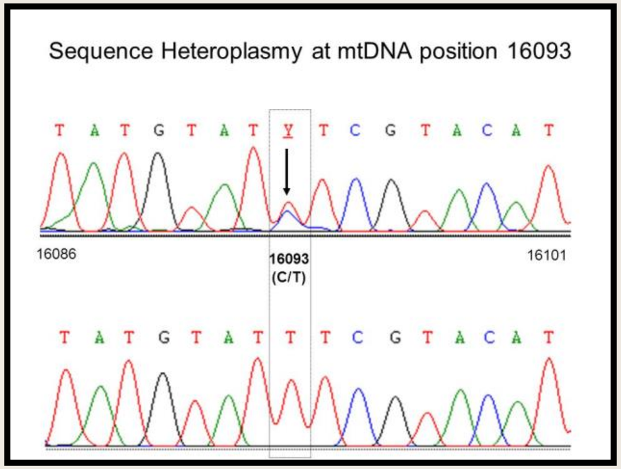
MT-DNA ANALYSIS – PILOT STUDY

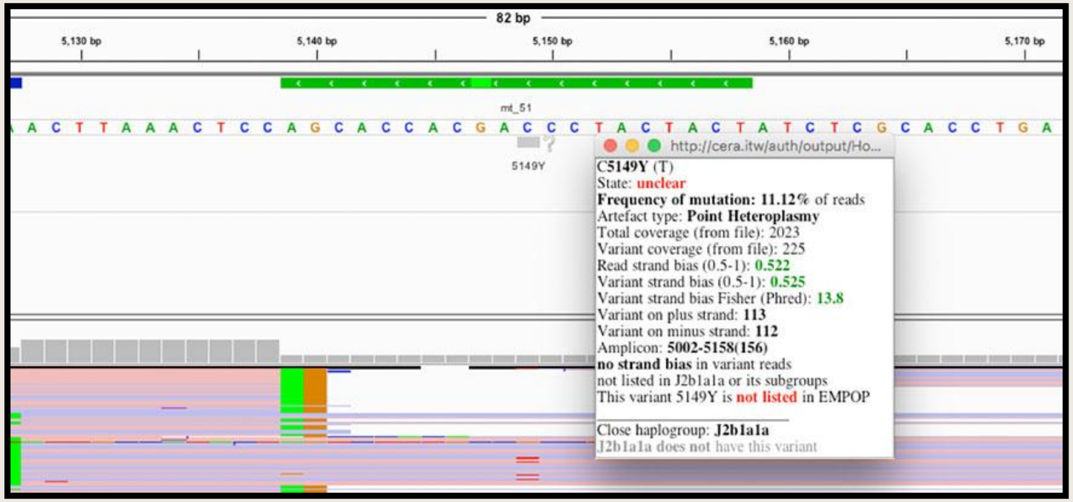
- ❑ **Comparison: non-concordance**
  - 58 of 58 samples incorrect calling of cytosine homopolymeric regions in HVI and HVII
  - Non-SWGDAM nomenclature used by Ion Reporter
    - i.e. Sample 49 variants “16187T 16188G 16189C” reported as “16187 TGC”
  - ~~1 missed insertion 291.1(non c repeat region)~~
  - ~~3 missed deletions at 249~~
  - ~~1 sample with missed variants at 263, 295~~
- ❑ **Further work**
  - Re-run on second chip
  - IGV analysis



MT-DNA ANALYSIS – PILOT STUDY

- ❑ **Things to consider**
  - % of heteroplasmy
    - Reporting thresholds
    - Inheritance of %
  - Length vs point heteroplasmy
  - Detection of sequencing errors
- ❑ **MPS**
  - Time effective
  - Increased coverage
- ❑ **Bioinformatics**
  - Better tools needed





C5149Y (T)
State: **unclear**
Frequency of mutation: 11.12% of reads
Artefact type: **Point Heteroplasmy**
Total coverage (from file): 2023
Variant coverage (from file): 225
Read strand bias (0.5-1): **0.522**
Variant strand bias (0.5-1): **0.525**
Variant strand bias Fisher (Phred): **13.8**
Variant on plus strand: **113**
Variant on minus strand: **112**
Amplicon: **5002-5158(156)**
no strand bias in variant reads
not listed in J2b1a1a or its subgroups
This variant 5149Y is **not listed** in EMPOP

Close haplogroup: **J2b1a1a**
J2b1a1a does not have this variant

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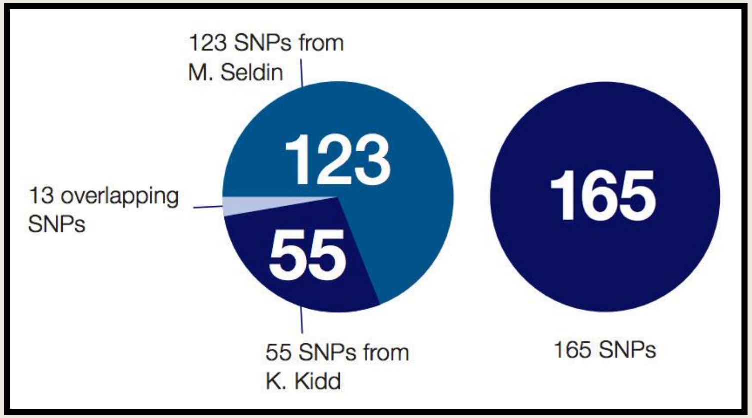
ID MARKERS – PILOT STUDY

- **Missing persons and cold case investigations**
  - Have ~ 40 UHRs in our care
  - Varying success of DNA profiling (STR and/or mtDNA)
- **DNA to predict physical appearance**
  - EVCs; e.g. eye and hair colour
- **DNA to predict ancestry**
  - BGA; e.g. European, Asian
- **Ion AmpliSeq™ ID panels**
  - 30 of the UHR cases
  - Two control cases (long term MP cases IDed using DNA matching)



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ID MARKERS – PILOT STUDY

Case 1

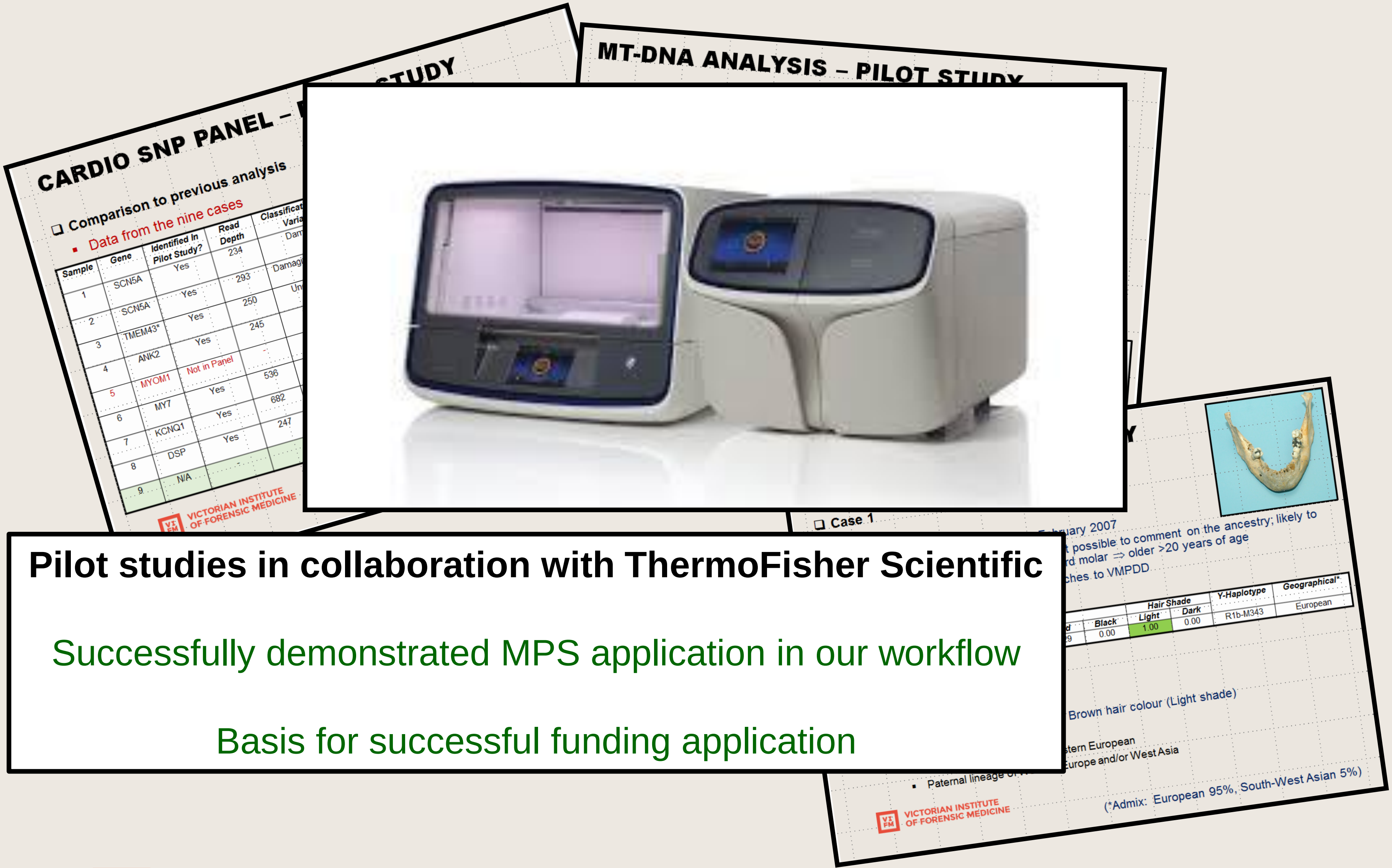
- UHR
  - Jaw bone located at a beach in February 2007
  - ***Anthropology***: absence cranium not possible to comment on the ancestry; likely to have been female; presence of a third molar ⇒ older >20 years of age
  - Conventional DNA profiling; No matches to VMPDD
- Phenotypic and ancestry analysis

Eye Colour			*Hair Colour*				*Hair Shade*		*Y-Haplotype*	*Geographical**
Blue	***Intermediate***	***Brown***	***Blond***	***Brown***	***Red***	***Black***	***Light***	***Dark***		
0.01	0.05	0.94	0.09	0.62	0.29	0.00	1.00	0.00	R1b-M343	European

- mtDNA profiling
  - HV (South- Eastern Europe)
- Information provided to MP Unit
  - Male with Brown eye colour and Brown hair colour (Light shade)
  - European ancestry
    - Maternal lineage of South-Eastern European
    - Paternal lineage of Western Europe and/or West Asia

(*Admix: European 95%, South-West Asian 5%)

MASSIVE PARALLEL SEQUENCING (MPS)



CARDIO SNP PANEL –

- ☐ Comparison to previous analysis
  - Data from the nine cases

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1	SCN5A	Yes	234	Dam
2	SCN5A	Yes	293	Damagi
3	TMEM43*	Yes	250	Un
4	ANK2	Yes	245	
5	MYOM1	Not in Panel	-	
6	MY7	Yes	536	
7	KCNQ1	Yes	682	
8	DSP	Yes	247	
9	N/A	-		

STUDY

MT-DNA ANALYSIS – PILOT STUDY

☐ Case 1

February 2007

t possible to comment on the ancestry; likely to

rd molar ⇒ older >20 years of age

ches to VMPDD

d	Black	Hair Shade Light	Dark	Y-Haplotype	Geographical*
9	0.00	1.00	0.00	R1b-M343	European

Brown hair colour (Light shade)

stern European

urope and/or West Asia

- Paternal lineage

(*Admix: European 95%, South-West Asian 5%)

Pilot studies in collaboration with ThermoFisher Scientific

Successfully demonstrated MPS application in our workflow

Basis for successful funding application

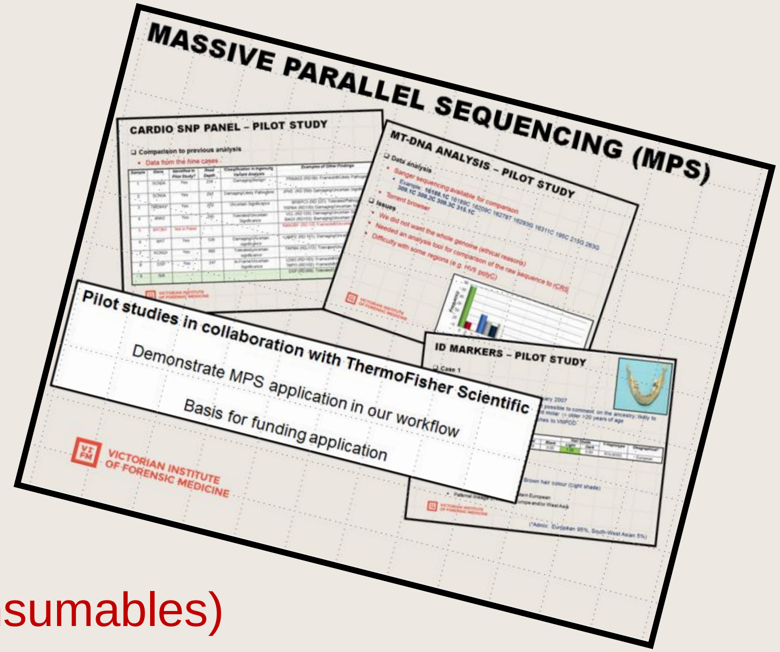
MPS VALIDATION

❑ **Where to begin?**

- Genetic screening
- Mitochondrial DNA analysis
- BGA and EVC analysis

❑ **For consideration**

- Limited resources (staff and funding for consumables)
- Build on existing expertise
- Uptake once validated



MASSIVE PARALLEL SEQUENCING (MPS)

CARDIO SNP PANEL – PILOT STUDY

❑ Comparison to previous analysis

- Data from the nine cases

MT-DNA ANALYSIS – PILOT STUDY

❑ Data analysis

- Sanger sequencing available for comparison
  - Example: 16188.1C 16189C 16209C 16278T 16293G 16311C 195C 215G 263G 309.1C 309.2C 309.3C 315.1C
- Torrent browser

❑ Issues

- We did not want the whole genome (ethical reasons)
- Needed an analysis tool for comparison of the raw sequence to rCRS
- Difficulty with some regions (e.g. HVII polyC)

ID MARKERS – PILOT STUDY

❑ Case 1

Pilot studies in collaboration with ThermoFisher Scientific

Demonstrate MPS application in our workflow

Basis for funding application

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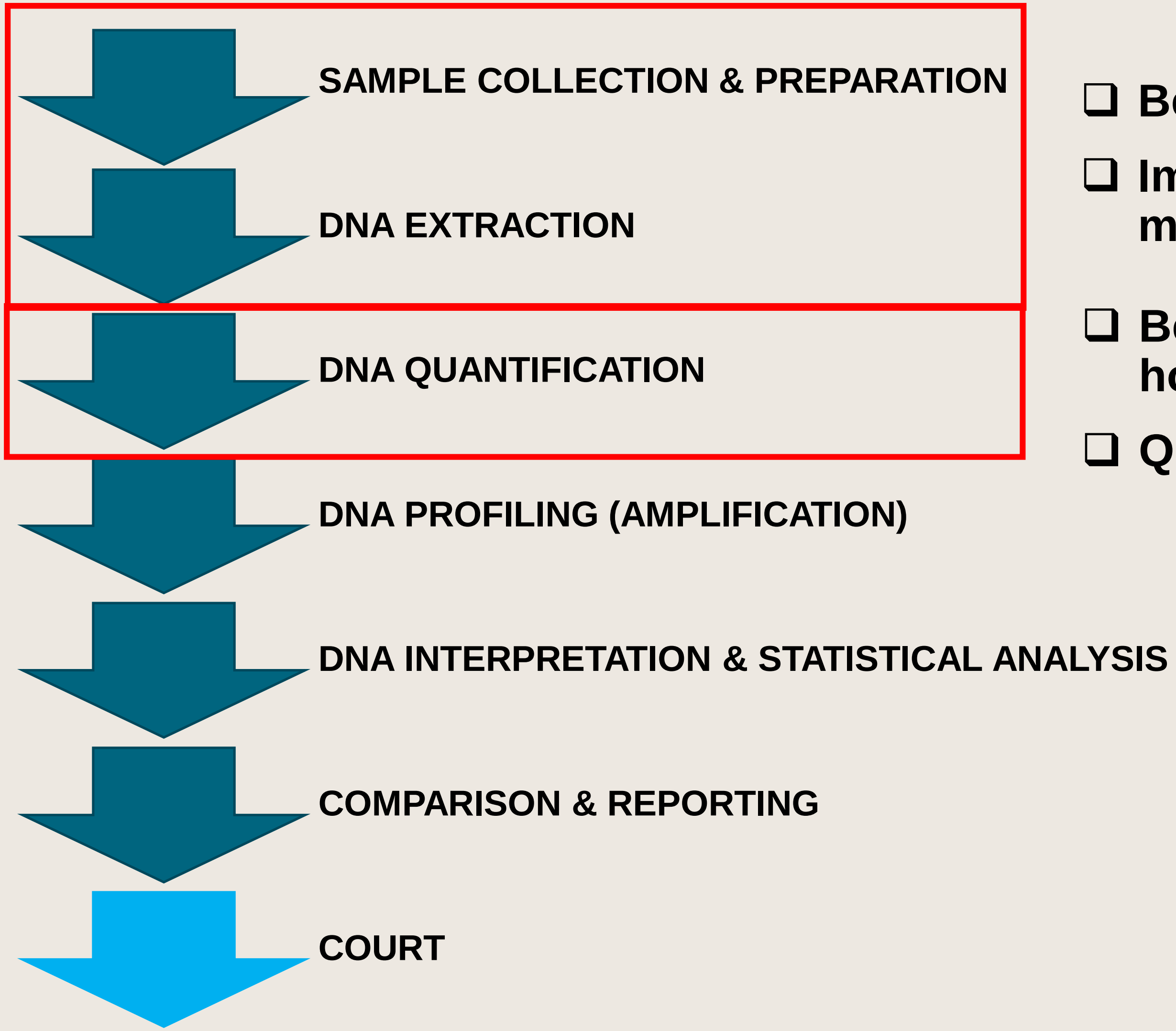
DNA ANALYSIS PIPELINE



SAMPLE COLLECTION & PREPARATION

DNA EXTRACTION

DNA QUANTIFICATION

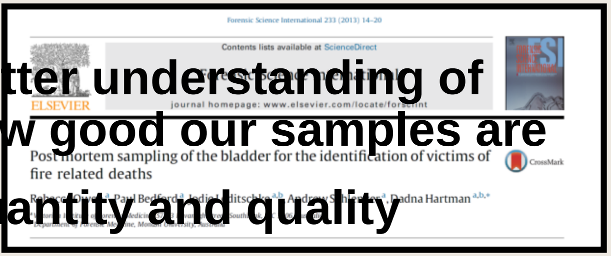
DNA PROFILING (AMPLIFICATION)

DNA INTERPRETATION & STATISTICAL ANALYSIS

COMPARISON & REPORTING

COURT

- ☐ **Better sample types**
- ☐ **Improved extraction methods**
- ☐ **Better understanding of how good our samples are**
- ☐ **Quantity and quality**



Forensic Science International 233 (2013) 14–20

Contents lists available at ScienceDirect

Forensic Science International

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

ELSEVIER

Post mortem sampling of the bladder for the identification of victims of fire related deaths

Rebecca Owen[a], Paul Bedford[a], Jodie Leditschke[a,b], Andrew Schlenker[a], Dadna Hartman[a,b,*]

[a] Victorian Institute of Forensic Medicine, 57–83 Kavanagh Street, Southbank, VIC 3006, Australia
[b] Department of Forensic Medicine, Monash University, Australia

Forensic Science International 258 (2016) 1–10

Contents lists available at ScienceDirect

Forensic Science International

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

ELSEVIER

Toenails as an alternative source material for the extraction of DNA from decomposed human remains

Andrew Schlenker[a], Katelyn Grimble[a], Arani Azim[a], Rebecca Owen[a], Dadna Hartman[a,b,*]

[a] Victorian Institute of Forensic Medicine, 65 Kavanagh St, Southbank, VIC 3006, Australia
[b] Department of Forensic Medicine, Monash University, Australia

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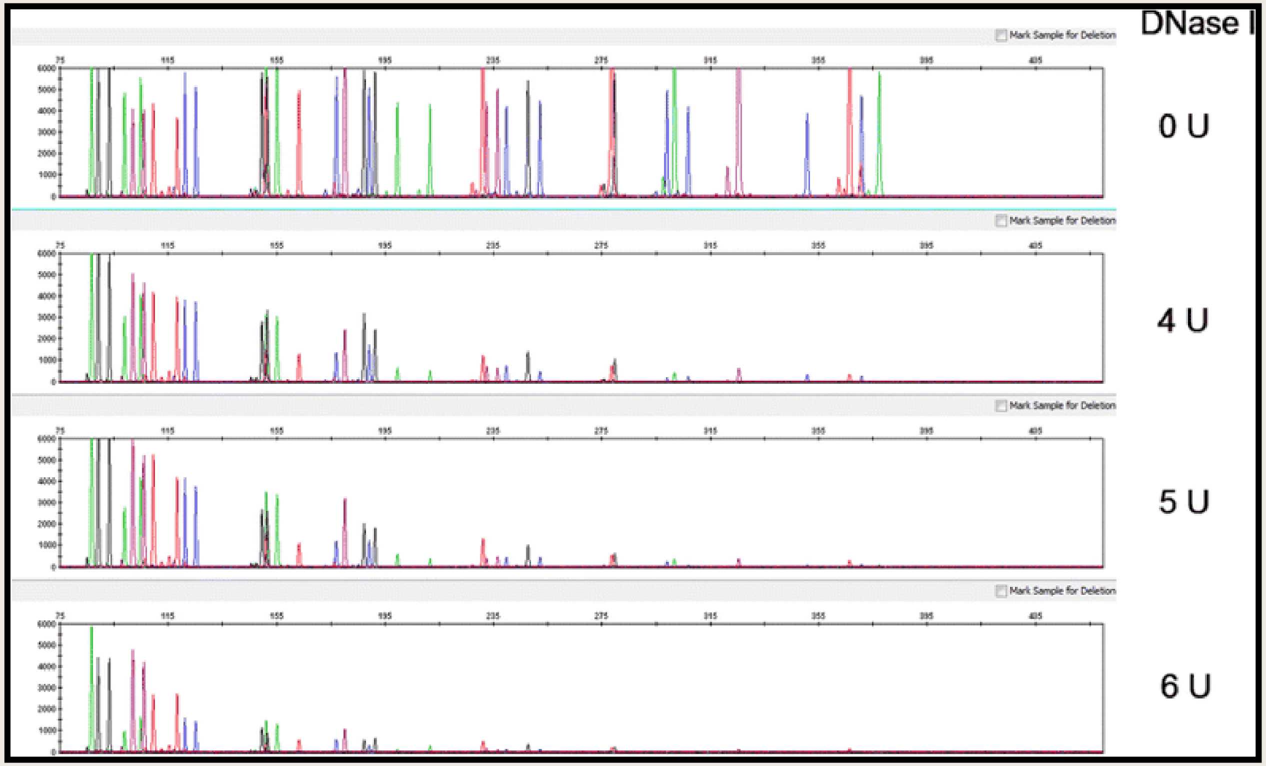
QUANTITY AND QUALITY OF N-DNA

- **Quantifiler HP kit**
  - Quantitative and qualitative assessment of total human DNA
- **Casework**
  - How degraded is my sample?
  - Include this in the decision matrix used to determine our amplification strategy with GlobalFiler kit



"Developmental validation of GlobalFiler™ PCR amplification kit: a 6-dye multiplex assay designed for amplification of casework samples" International J. Legal Med. (2018) 132: 1555

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DEGRADATION

❑ Experimental design

- 10 x 11 x 10 experiment
  - 10 samples with 11 descending concentrations with 10 increasing degradation levels

		Concentration (ng/ul)										
		0.067	0.05-0.067	0.03-0.05	0.02-0.03	0.012-0.02	0.077-0.012	0.005-0.077	0.032-0.005	0.002-0.0032	0.0014-0.002	<0.0014
Degradation Index	<1	C1D1	C2D1	C3D1	C4D1	C5D1	C6D1	C7D1	C8D1	C9D1	C10D1	C11D1
	1-1.25	C1D2	C2D2	C3D2	C4D2	C5D2	C6D2	C7D2	C8D2	C9D2	C10D2	C11D2
	1.25-1.5	C1D3	C2D3	C3D3	C4D3	C5D3	C6D3	C7D3	C8D3	C9D3	C10D3	C11D3
	1.5-2	C1D4	C2D4	C3D4	C4D4	C5D4	C6D4	C7D4	C8D4	C9D4	C10D4	C11D4
	2-3	C1D5	C2D5	C3D5	C4D5	C5D5	C6D5	C7D5	C8D5	C9D5	C10D5	C11D5
	3-5	C1D6	C2D6	C3D6	C4D6	C5D6	C6D6	C7D6	C8D6	C9D6	C10D6	C11D6
	5-7.5	C1D7	C2D7	C3D7	C4D7	C5D7	C6D7	C7D7	C8D7	C9D7	C10D7	C11D7
	7.5-10	C1D8	C2D8	C3D8	C4D8	C5D8	C6D8	C7D8	C8D8	C9D8	C10D8	C11D8
	10-20	C1D9	C2D9	C3D9	C4D9	C5D9	C6D9	C7D9	C8D9	C9D9	C10D9	C11D9
	>20	C1D10	C2D10	C3D10	C4D10	C5D10	C6D10	C7D10	C8D10	C9D10	C10D10	C11D10

- Test the performance of GlobalFiler kit
  - Ideal (input concentration with no sample degradation)
  - Additional 109 concentrations-degradation categories
- Genemapper ID-X Software (v1.4) with 45RFU as the analytical threshold

DEGRADATION

❑ Degradation matrix

- GlobalFiler amp worksheet
  - Input small and large autosomal data
  - Sample is assigned C-D category
  - Most appropriate amp strategy based on required completeness of profile entered (e.g. 75%)
  - The expected % completeness at the selected amplification strategy
- Verification
  - Quantification and profiling data from all casework samples processed since the implementation of GlobalFiler kit
  - High rate of correct predictions (>98%) at 1 x amp

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Minimum data recovery 75%

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
D1	x1	x1	x1	x1	x1	x2	x2	x2	x4	x4
D2	x1	x1	x1	x2	x2	x2	x2	x4	x4	x4
D3	x1	x1	x1	x2	x2	x2	x4	x4	x4	x4
D4	x1	x1	x2	x2	x2	x2	x4	x4	x4	x4
D5	x1	x2	x2	x2	x2	x4	x4	x4	x4	x4
D6	x2	x2	x2	x4	x4	x4	x4	x4	x4	x4
D7	x2	x2	x4	x4	x4	x4	x4	x4	x4	x4
D8	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D9	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D10	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4

Minimum data recovery 90%

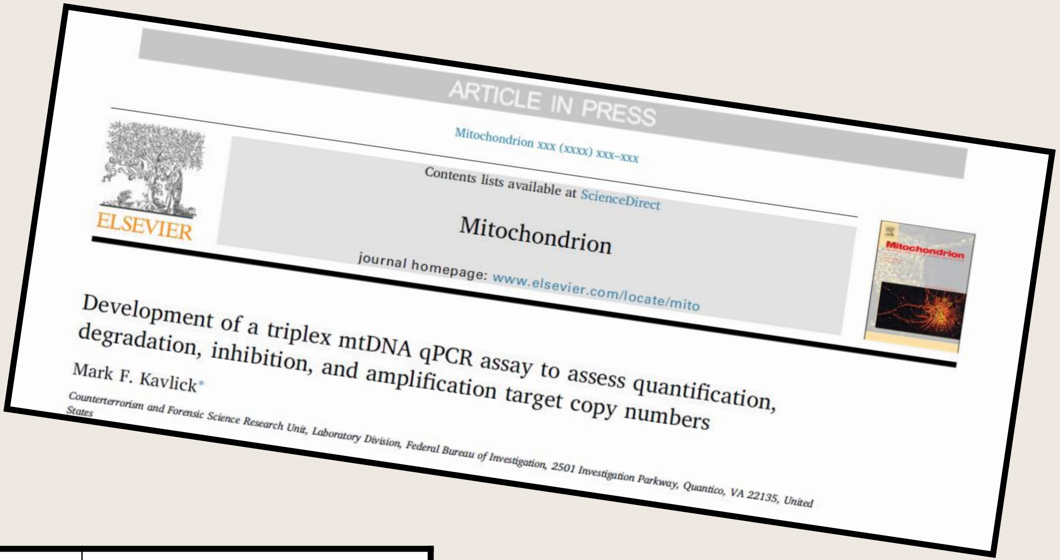
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
D1	x1	x1	x1	x1	x2	x2	x2	x4	x4	x4
D2	x1	x1	x1	x2	x2	x2	x4	x4	x4	x4
D3	x1	x1	x1	x2	x2	x2	x4	x4	x4	x4
D4	x1	x2	x2	x2	x4	x4	x4	x4	x4	x4
D5	x2	x2	x2	x2	x4	x4	x4	x4	x4	x4
D6	x2	x4	x4	x4	x4	x4	x4	x4	x4	x4
D7	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D8	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D9	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D10	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4

Minimum data recovery 100%

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
D1	x1	x1	x1	x2	x2	x2	x4	x4	x4	x4
D2	x1	x2	x2	x2	x2	x4	x4	x4	x4	x4
D3	x1	x2	x2	x2	x2	x4	x4	x4	x4	x4
D4	x1	x2	x2	x4	x4	x4	x4	x4	x4	x4
D5	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D6	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D7	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D8	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D9	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4
D10	x4	x4	x4	x4	x4	x4	x4	x4	x4	x4

QUANTITY AND QUALITY OF MT-DNA

- ❑ **Quantification kit**
  - None commercially available
- ❑ **In-house quantification**
  - Based on published method



ARTICLE IN PRESS

Mitochondrion xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

ELSEVIER

Mitochondrion

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

Development of a triplex mtDNA qPCR assay to assess quantification, degradation, inhibition, and amplification target copy numbers

Mark F. Kavlick*

Counterterrorism and Forensic Science Research Unit, Laboratory Division, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135, United States

Sample	*Nuclear DNA (ng/µL)*	*Mito DNA (copies/µL)*
1	0.00068	16,883
2	Undetermined	0 (Inhibited)
3	0.00096	1,179
4	Undetermined	368
5	0.00068	676
6	Undetermined	91
7	0.011	12,941

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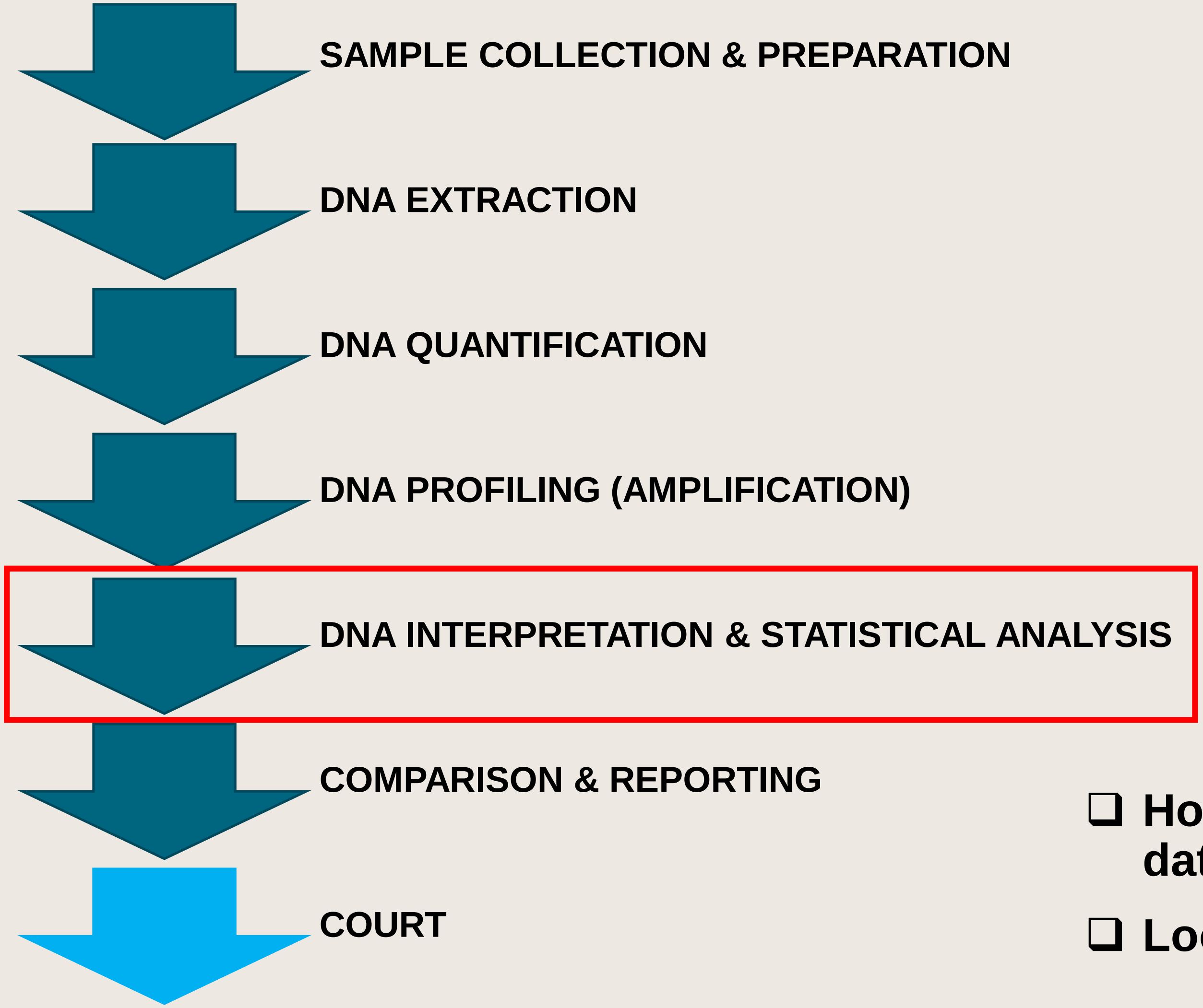
DNA ANALYSIS PIPELINE



SAMPLE COLLECTION & PREPARATION

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COURT

- ☐ **How do we analyse MPS data generated?**
- ☐ **Looking for a plug & play**

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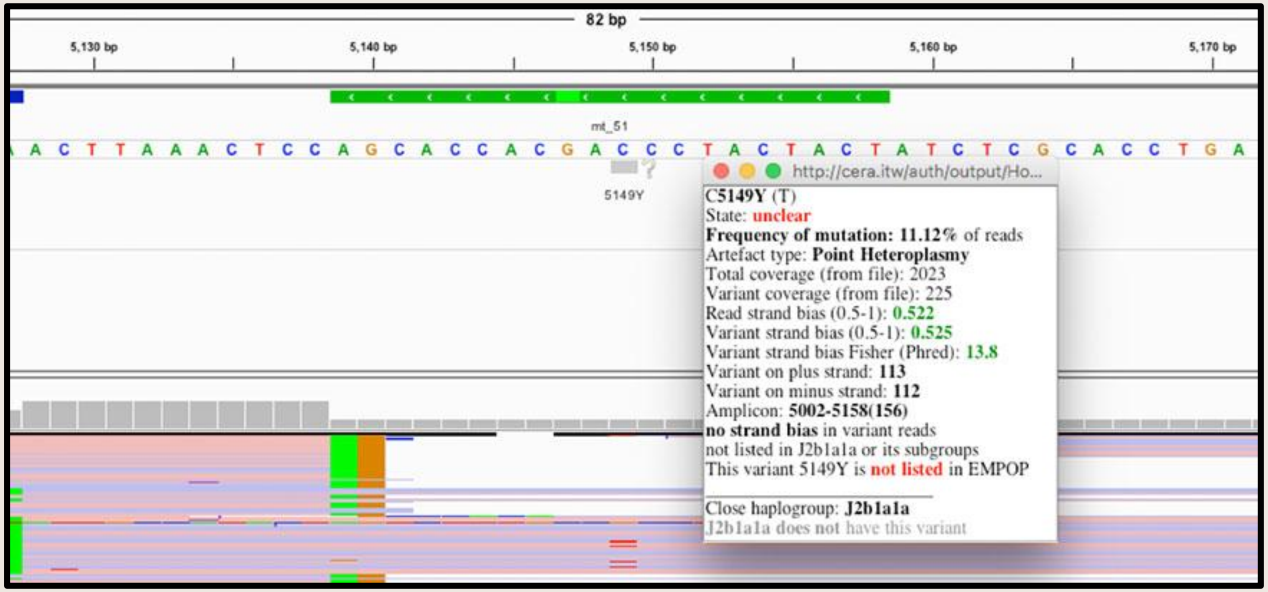
ANALYSIS TOOLS – CONVERGE™ SOFTWARE

converge

- ❑ **Converge™**
  - MPS data analysis
    - Mitochondrial DNA (mtDNA)
    - Ancestry (biogeographic) SNPs
    - Identity SNPs
    - STR markers
  - CE to MPS comparison of STR profiles
  - Kinship
  - Case management



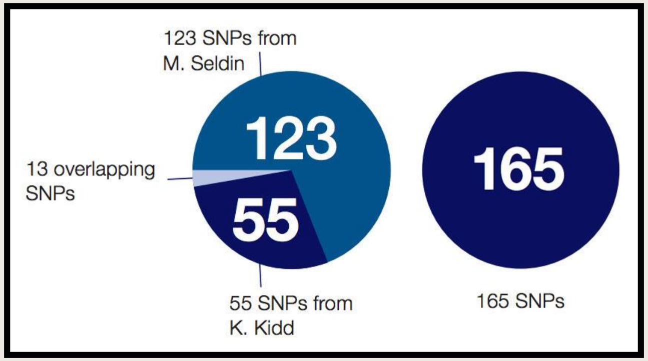
C5149Y (T)
State: **unclear**
Frequency of mutation: 11.12% of reads
Artefact type: **Point Heteroplasmy**
Total coverage (from file): 2023
Variant coverage (from file): 225
Read strand bias (0.5-1): **0.522**
Variant strand bias (0.5-1): **0.525**
Variant strand bias Fisher (Phred): 13.8
Variant on plus strand: **113**
Variant on minus strand: **112**
Amplicon: **5002-5158(156)**
no strand bias in variant reads
not listed in J2b1a1a or its subgroups
This variant 5149Y is **not listed** in EMPOP

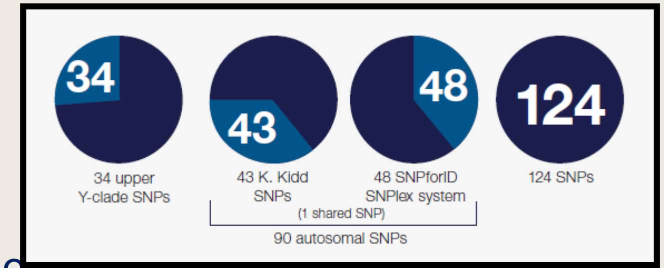
Close haplogroup: **J2b1a1a**
J2b1a1a does not have this variant

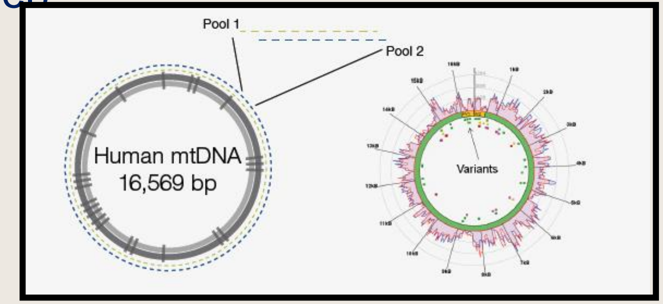
UHR CASES

☐ **MPS analysis**

- 8 UHR cases
  - Varying degrees of DNA quantity and quality
- Panels
  - Precision ID Ancestry Panel
  - Precision ID Identity Panel
  - DNA Phenotyping Panel (Ion Ampliseq™ custom pane
  - Precision ID mtDNA Control Region Panel
- Libraries & Sequencing
  - Library construction on Ion Chef™ System
  - Template preparation on Ion Chef System
  - Sequencing on Ion 530™ chip on Ion S5™ System
- Analysis
  - Converge Software
  - IrisPlex (EVC data)







Sample	*Nuclear DNA (ng/µL)*	*Mito DNA (copies/µL)*	*Median Read Depth*
1	0.00068	16,883	15,931
2	Undetermined	0 (Inhibited)	23,297
3	0.00096	1,179	20,290
4	Undetermined	368	26,823
5	0.00068	676	0
6	Undetermined	91	27,478
7	0.011	12,941	19,574
8	0.007	Not Available	18,408

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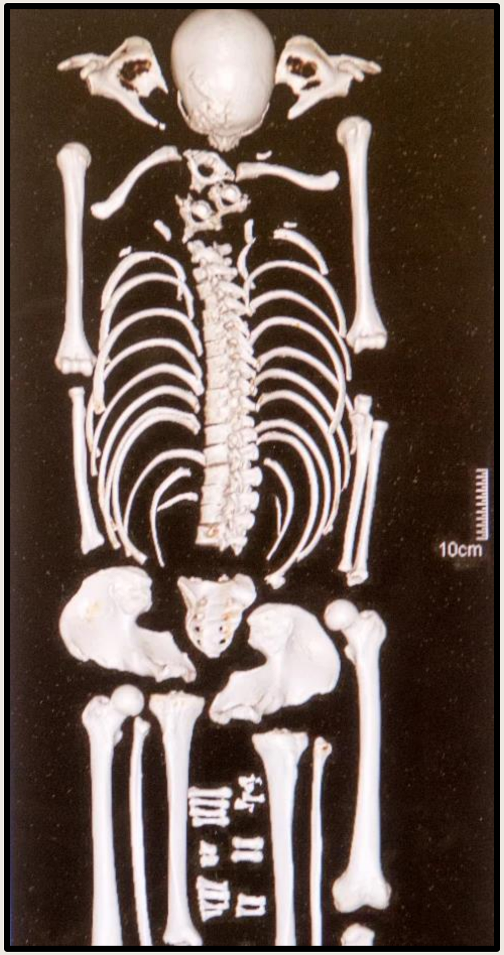
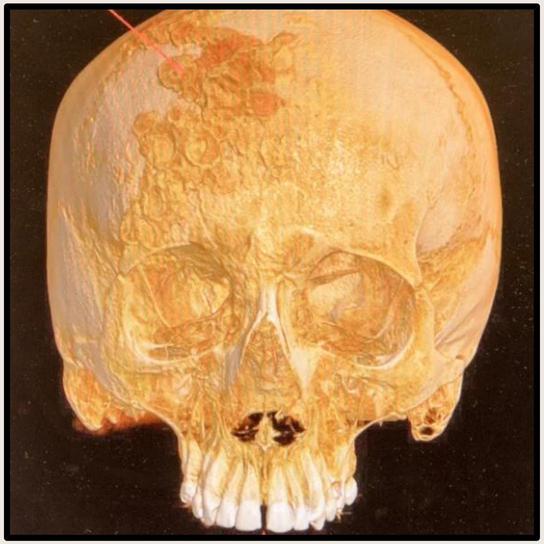
UHR CASES

- ❑ **Sandy Point Skeleton**
  - UHR
    - December 2017
    - Sandy Point coast line; near Wilsons Promontory in Victoria
    - Complete skeleton; no fractures; few bones missing;
    - No soft tissue
    - Distinctive dental work (a gold filling in his front tooth)
    - ***Anthropology***: Caucasoid male; in his 20s
    - ***Conventional DNA profiling:*** nDNA and mtDNA profiling
    - No matches to VMPDD





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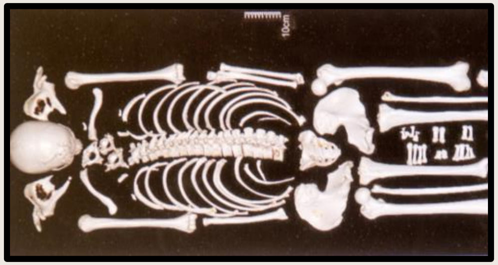
UHR CASES

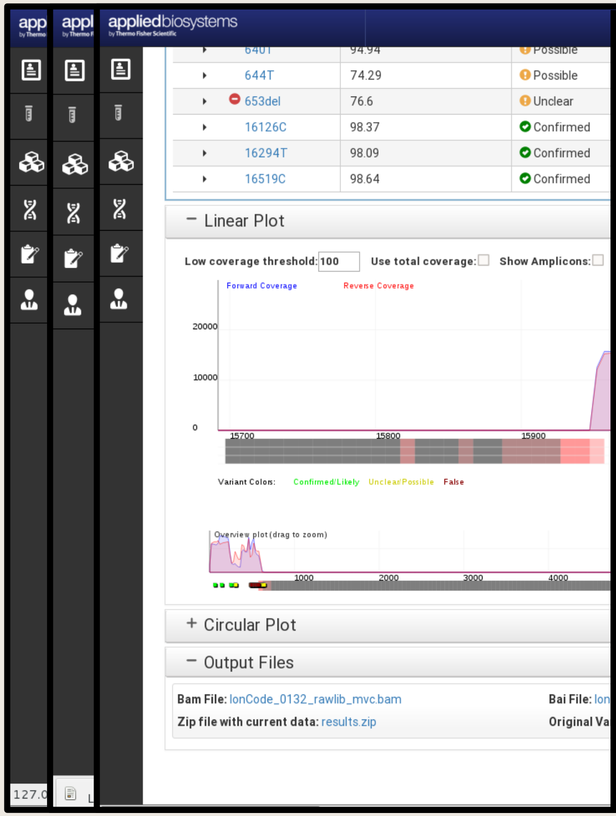


☐ Sandy Point Skeleton

- MPS – mtDNA analysis
  - T2
    - Maternal lineage: West Eurasia

HVI (16024-16389)	16126C 16294T
HVII (51-388)	73G 152C 263G 315.1C

HV	16126C 16294T 16519C 73G 152C 263G 315.1C



644T	74.29	Possible
653del	76.6	Unclear
16126C	98.37	Confirmed
16294T	98.09	Confirmed
16519C	98.64	Confirmed

− Linear Plot

Low coverage threshold: 100 Use total coverage: ☐ Show Amplicons: ☐

+ Circular Plot

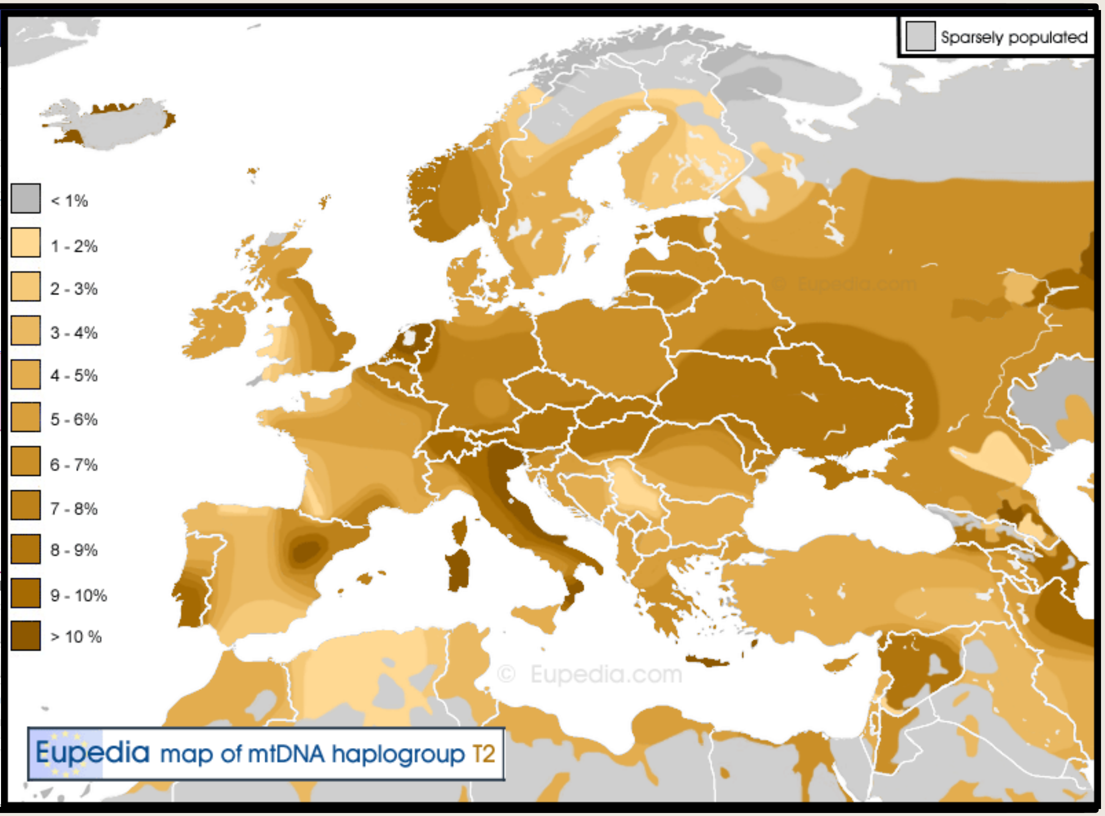
− Output Files

Bam File: IonCode_0132_rawlib_mvc.bam

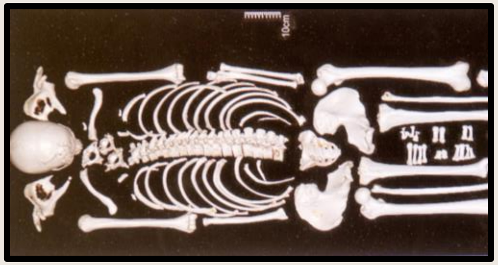
Bai File: Ion

Zip file with current data: results.zip

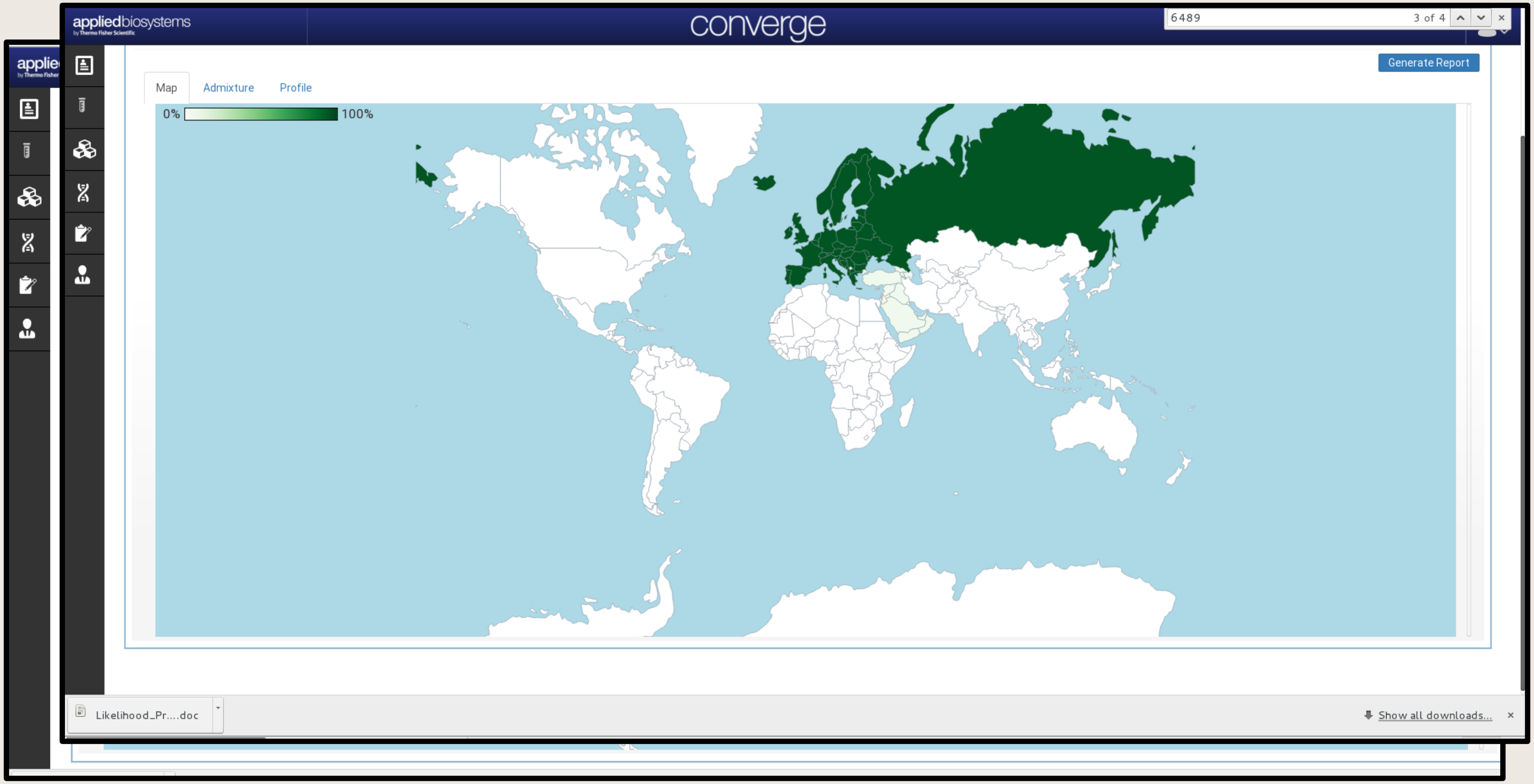
Original Va



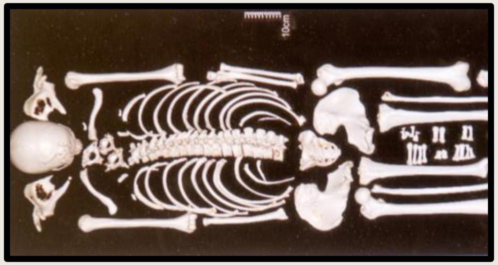
UHR CASES



- ❑ **Sandy Point Skeleton**
  - BGA analysis
    - Admix: 95% Europe; 5% South-West Asia

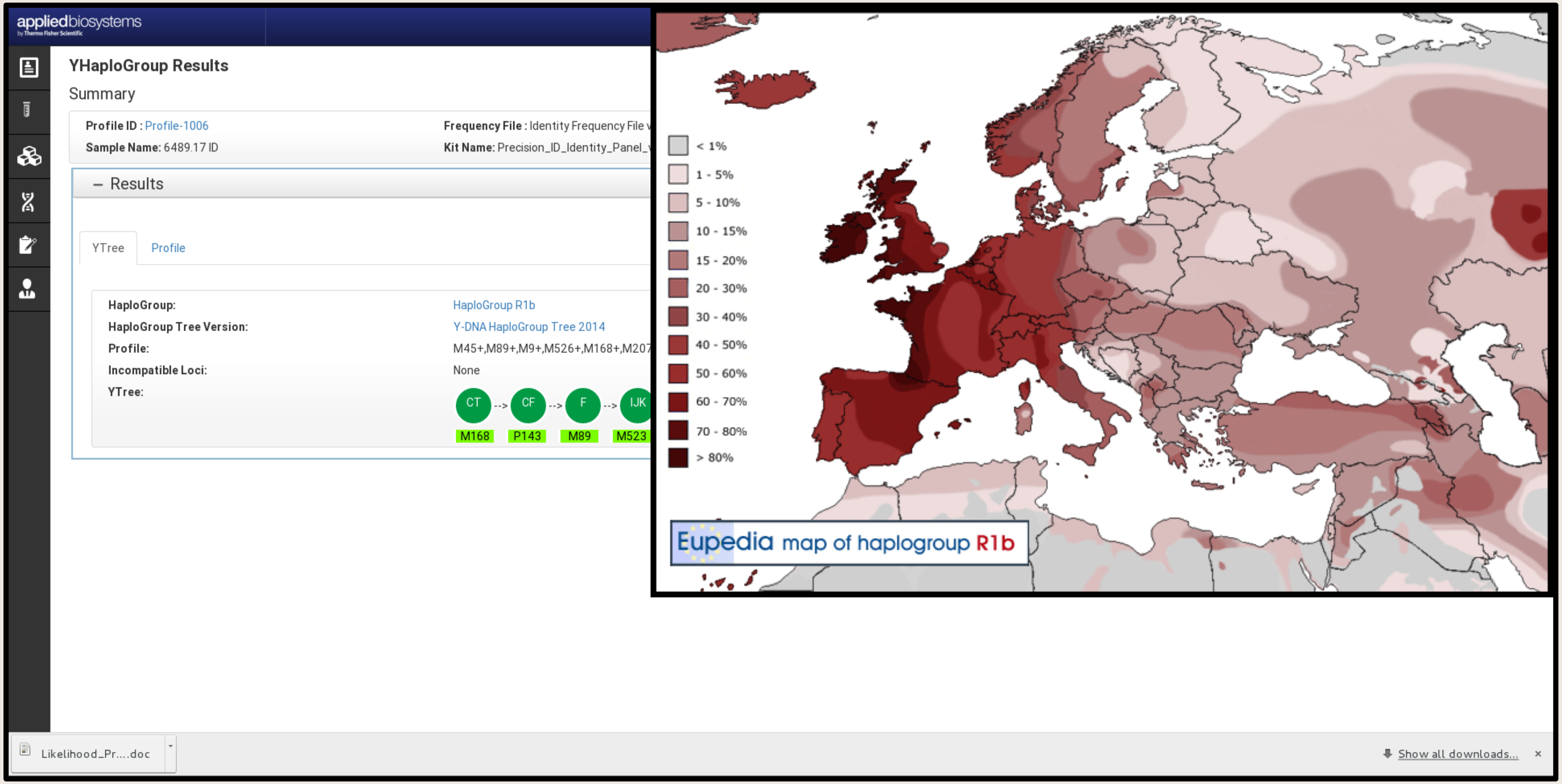


UHR CASES



❑ Sandy Point Skeleton

- Y Haplogroup
  - R1b
    - Paternal lineage: Western Europe



YHaploGroup Results

Summary

Profile ID : Profile-1006

Sample Name: 6489.17 ID

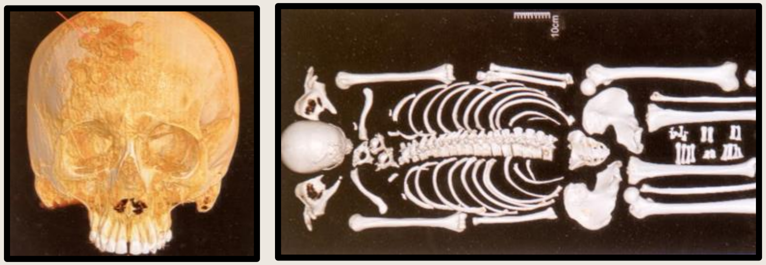
Frequency File : Identity Frequency File

Kit Name: Precision_ID_Identity_Panel_

– Results

HaploGroup:	HaploGroup R1b
HaploGroup Tree Version:	Y-DNA HaploGroup Tree 2014
Profile:	M45+,M89+,M9+,M526+,M168+,M207
Incompatible Loci:	None
YTree:	CT --> CF --> F --> IJK M168 P143 M89 M523

UHR CASES



- ☐ **Sandy Point Skeleton**
  - UHR
    - Complete skeleton; no fractures; few bones missing;
    - Distinctive dental work (a gold filling in his front tooth)
    - ***Anthropology***: Caucasoid male; in his 20s
    - ***Conventional DNA profiling:*** nDNA and mtDNA profiling
  - EVC & BGA

Eye Colour			*Hair Colour*				*Hair Shade*		*Y-Haplotype*	*Geographical**
Blue	***Intermediate***	***Brown***	***Blond***	***Brown***	***Red***	***Black***	***Light***	***Dark***		
0.55	0.15	0.30	0.02	0.56	0.00	0.42	0.16	0.84	R1b	European

- mtDNA profiling

HV	73G 152C 263G 315.1C 16126C 16294T 16519C

  - T2 (West Eurasia)

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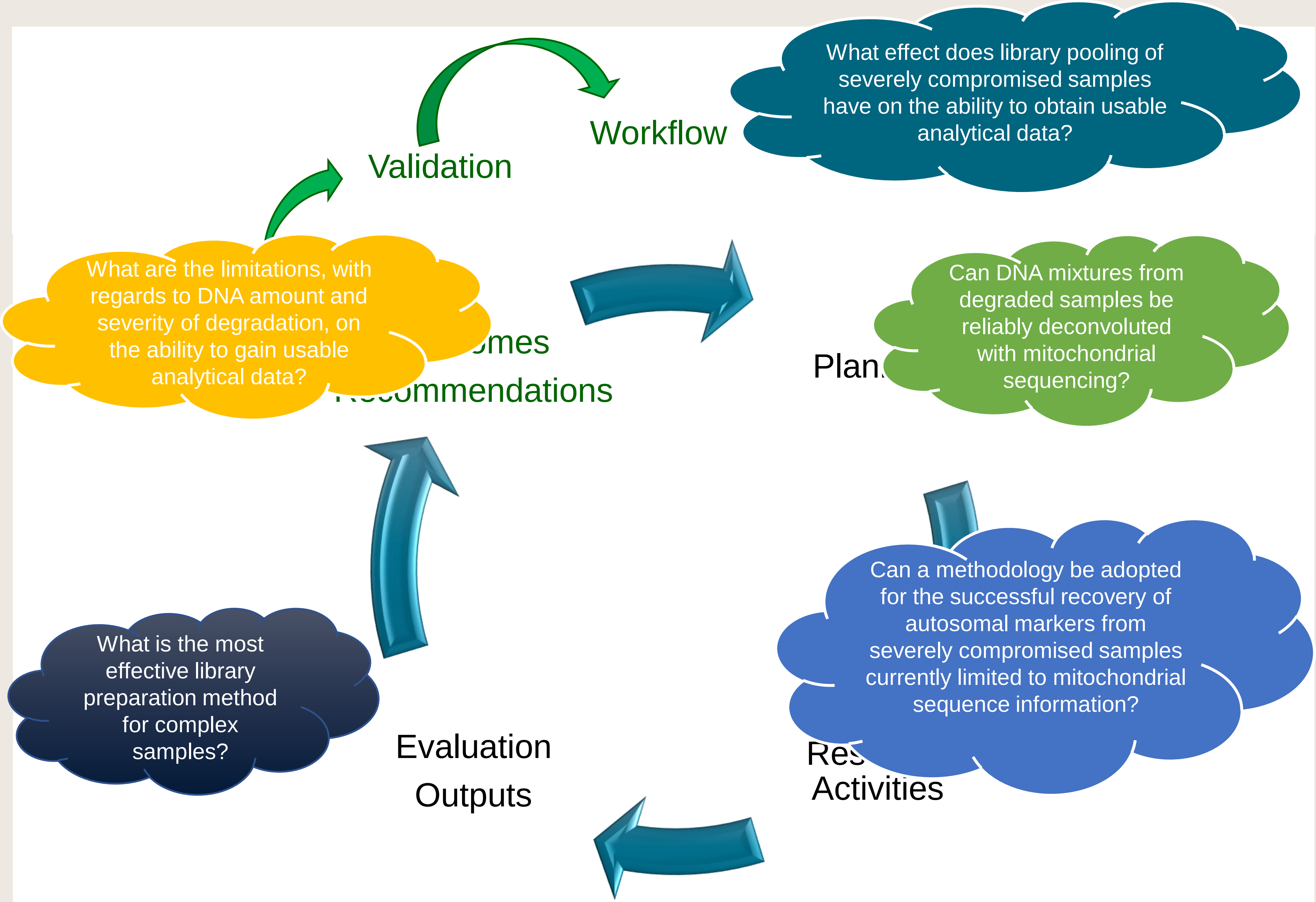
(*Admix: European 95%, South-West Asian 5%)

NEXT



Validation

Workflow

What effect does library pooling of severely compromised samples have on the ability to obtain usable analytical data?

What are the limitations, with regards to DNA amount and severity of degradation, on the ability to gain usable analytical data?

omes
ecommendations

Plan

Can DNA mixtures from degraded samples be reliably deconvoluted with mitochondrial sequencing?

Can a methodology be adopted for the successful recovery of autosomal markers from severely compromised samples currently limited to mitochondrial sequence information?

Res
Activities

Evaluation
Outputs

What is the most effective library preparation method for complex samples?

ACKNOWLEDGMENTS

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Linda Benton
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Ashil Davawala
Dadna Hartman
Kaitlyn Hart
Andrew Schlenker
Michelle Spiden
April Stock

Coroners Court of Victoria

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THANK YOU