15NIJ-22-GG-04395-DNAX
NIJ 2020-DQ-BX-0004

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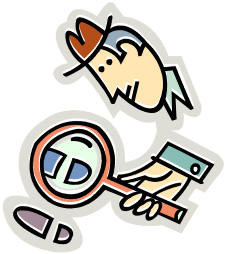

Advances in Mitogenome Sequencing for Forensic Laboratories

Technical Leader Meeting, ISHI
21 September 2023

https://sites.psu.edu/hollandresearch/

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ADVANCED REVIEW

WIREs FORENSIC SCIENCE WILEY

The time is now for ubiquitous forensic mtMPS analysis

Lauren C. Canale[1] | **Walther Parson**[1,2] | **Mitchell M. Holland**[1]

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[2]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria

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Edited by Michael Coble, Editor

Abstract
Mitochondrial (mt) DNA sequence analysis is useful for assessing ancestral origin and migration, identifying human remains, and examining evidentiary material in forensic casework. Conventional Sanger-type sequencing (STS) has been used for more than three decades to address these interests. This paper reviews the methodologies and merits of using a massively parallel sequencing (MPS) approach for mtDNA testing in forensic laboratories, as *The Time is Now for Ubiquitous Forensic mtMPS Analysis*.

This article is categorized under:
Forensic Biology > Haploid Markers
Forensic Biology > Forensic DNA Technologies

KEYWORDS
forensic DNA, massively parallel sequencing, mitochondrial DNA

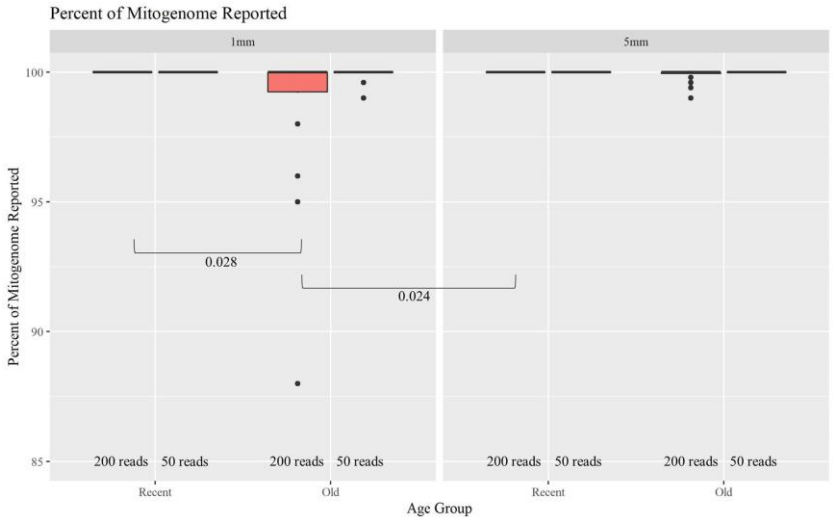
2021

genes 2022 MDPI

Article

Routine Mitogenome MPS Analysis from 1 and 5 mm of Rootless Human Hair

Lauren C. Canale [1], Jennifer A. McElhoe [1], Gloria Dimick [2], Katherine M. DeHeer [3], Jason Beckert [4] and Mitchell M. Holland [1,2,*]



CURRENT PROTOCOLS
A Wiley Brand

Massively Parallel Sequencing of the Mitogenome from Human Hair Shafts in Forensic Investigations

Jade T. Korber,[1] Lauren C. Canale,[2] and Mitchell M. Holland[1,3,4]

[1]Department of Biochemistry & Molecular Biology, Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania
[2]Department of Justice, Jan Bashinski DNA Laboratory, Richmond, California
[3]Mitotyping Technologies, State College, Pennsylvania
[4]Corresponding author: *mmh20@psu.edu*

Published in the Human Genetics section

2023

https://sites.psu.edu/hollandresearch/

CASE REPORT

Mitochondrial DNA:
State of Tennessee v. Paul Ware

By C. Leland Davis, ADA
District Attorney's Office, Chattanooga, TN

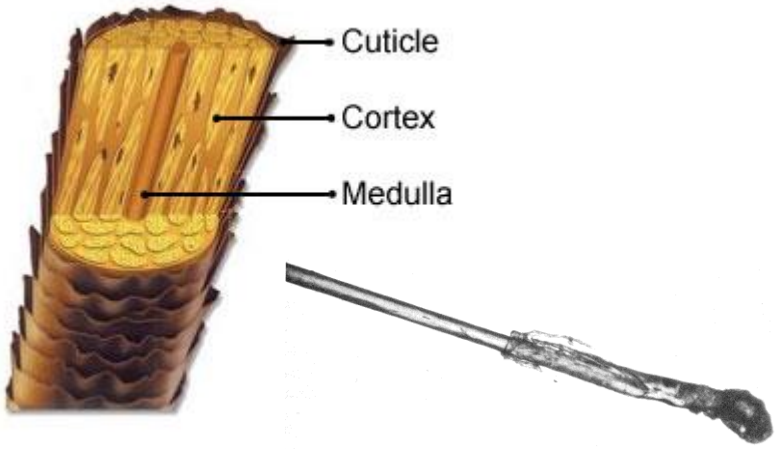
FBI 1996

Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II

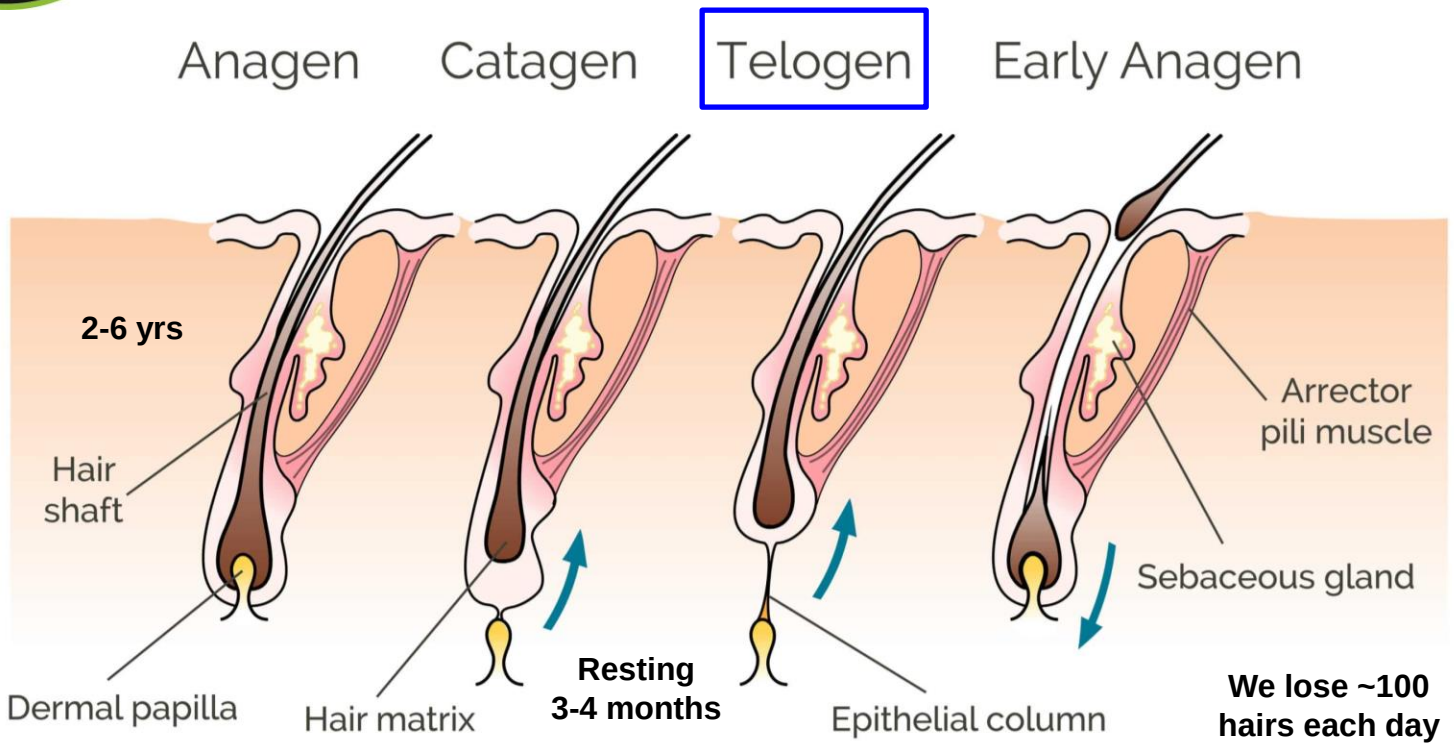
Pavel L. Ivanov[1], Mark J. Wadhams[2], Rhonda K. Roby[2], Mitchell M. Holland[2], Victor W. Weedn[2] & Thomas I. Parsons[2]

nature genetics volume 12 april 1996

mtDNA sequence analysis is a useful tool for testing **hair shafts** and old skeletal remains associated with criminal and identification cases



Hairs are the most common biological evidence type found at crime scenes



Shed hairs (telogen) represent up to 90% of evidentiary hairs

While chromosomal DNA typically accounts for >95% of the DNA content in hair shafts, the nucDNA is highly degraded with an average fragment length of 40-70 bps, more degraded than mtDNA

Table 11. Sequencing statistics for recent single hairs.

Extracts	# Unique Human Reads	# mtDNA Unique Reads	# Unique nuDNA Reads	% mtDNA/% nuDNA (bp)	% mtDNA/% nuDNA (Reads)
R1	34,909	1,865	33,044	11.95/88.05	5.3/94.7
R2	327,165	1,707	325,458	0.59/99.41	0.5/99.5
R3	42,308	2,287	40,021	9.29/90.71	5.4/94.6
R4	196,737	4,790	191,947	2.88/97.12	2.4/97.6
R5	94,969	10,997	83,972	14.08/85.92	11.6/88.4
R6	952,728	25,575	927,153	3.56/96.44	2.7/97.3

genes

MDPI

Article

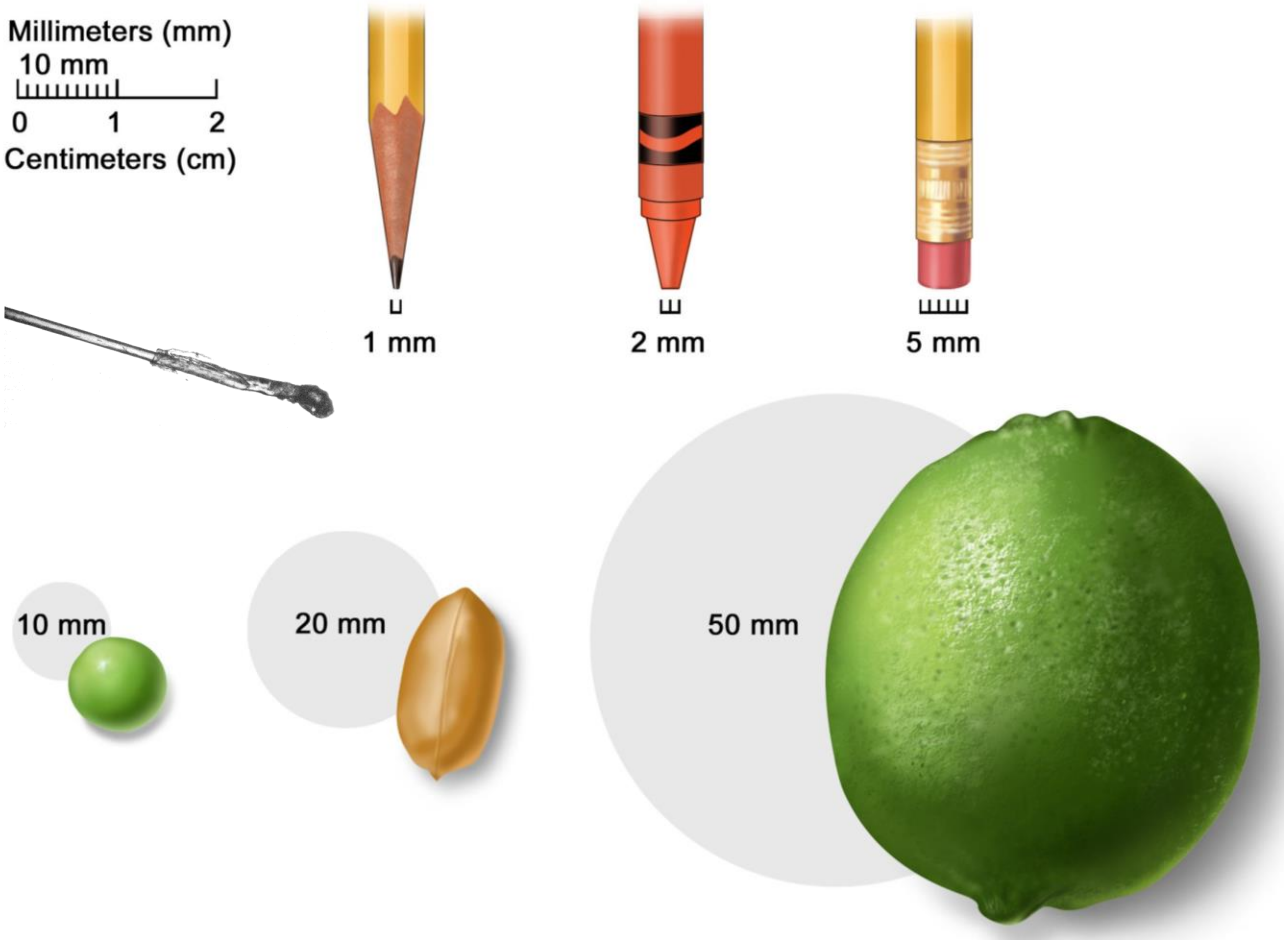
Fragmented Nuclear DNA is the Predominant Genetic Material in Human Hair Shafts

Michael D. Brandhagen [1,†], Odile Loreille [1,†], and Jodi A. Irwin [1,*]

DNA Support Unit, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135, USA; mdbrandhagen@fbi.gov (M.D.B.); oploreille@fbi.gov (O.L.)

Ed Green
UC Santa Cruz
Astrea

Routine mitoGenome MPS Analysis from 1-5 mm of Human Hair Shaft



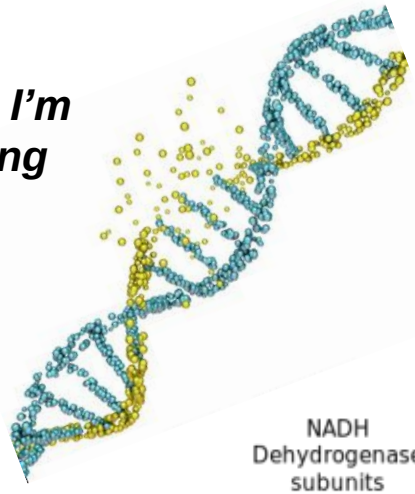
National Cancer Institute

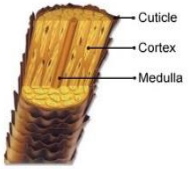
What most crime labs focus on

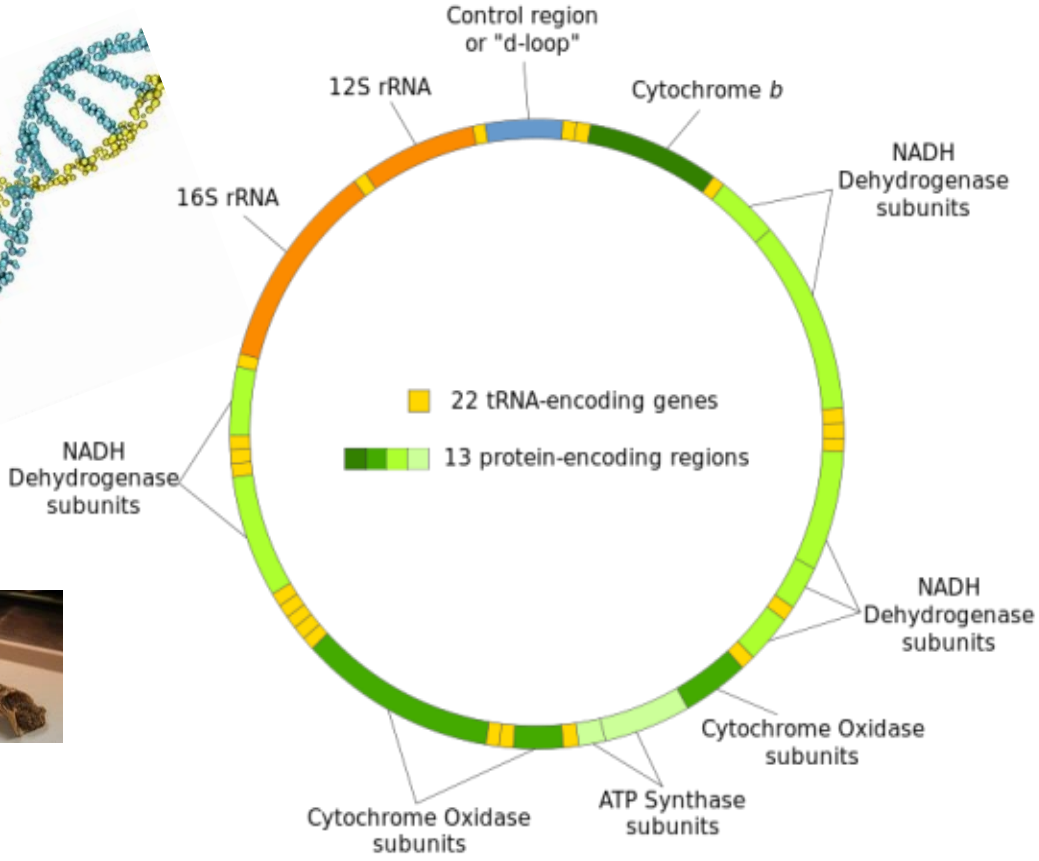
HV1/HV2 = 610 bps

CR = 1122 bps

Help me, I'm degrading



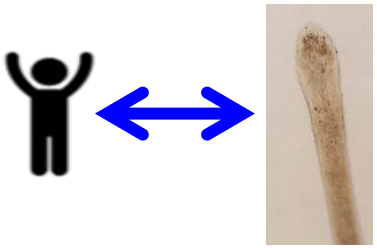




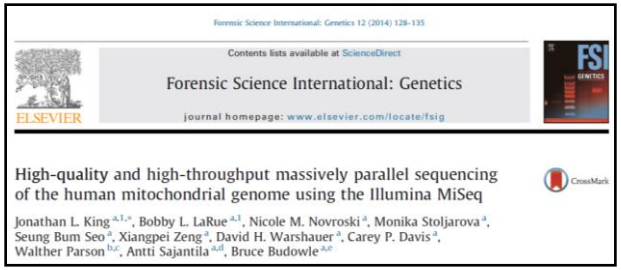
Where the field is likely to move

mitoGenome = ~16,569 bps

Better Discrimination Potential



Populations	*n*	HVI/HVII		mtGenome	
		RMP	GD	RMP	GD
AFA	87	2.42%	98.72%	1.31%	99.84%
CAU	83	3.12%	98.06%	1.20%	100.00%
HIS	113	3.33%	97.53%	0.98%	99.91%
Mean ± SD		2.96 ± 0.48%	98.10 ± 0.59%	1.16[c] ± 0.17%	99.91[d] ± 0.08%



Forensic Science International: Genetics 12 (2014) 128–135

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

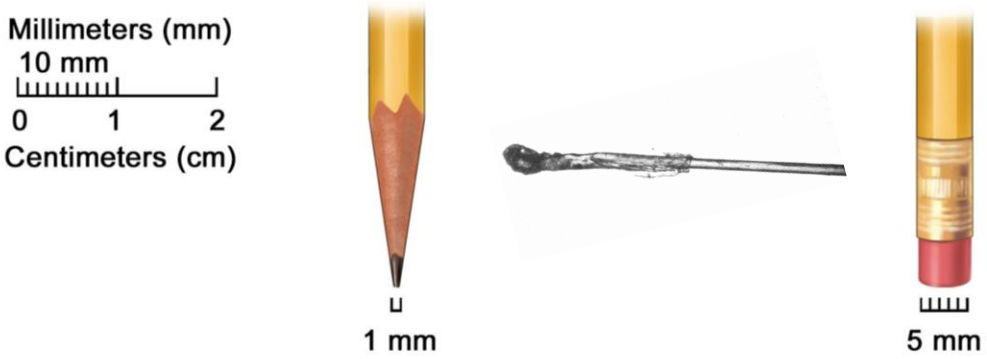
High-quality and high-throughput massively parallel sequencing of the human mitochondrial genome using the Illumina MiSeq

Jonathan L. King[a,1,*], Bobby L. LaRue[a,1], Nicole M. Novroski[a], Monika Stoljarova[a], Seung Bum Seo[a], Xiangpei Zeng[a], David H. Warshauer[a], Carey P. Davis[a], Walther Parson[b,c], Antti Sajantila[a,d], Bruce Budowle[a,e]

Amplification Approaches & Kits Available

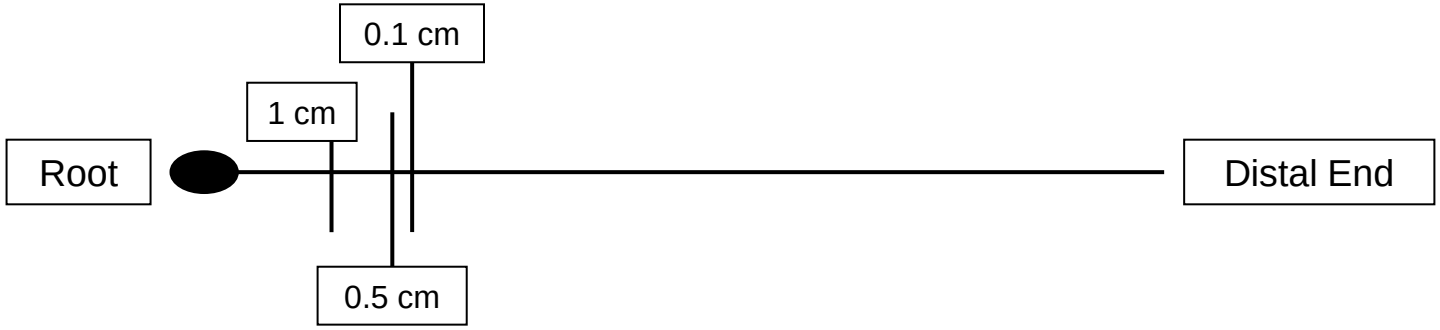
- Promega
  - PowerSeq CRM (control region, 1 multiplex, 144-237 bps)
  - **PowerSeq WGM (mitogenome, 1 multiplex of 161 amplicons averaging 167 bps, research product)**
- Verogen
  - ForenSeq mtDNA Control Region (2 multiplexes, 18 amplicons averaging 118 bps)
  - ForenSeq mtDNA Whole Genome (2 multiplexes, 245 amplicons averaging 131 bps)
- ThermoFisher
  - Precision ID mtDNA Control Region Panel (2 multiplexes)
  - Precision ID mtDNA Whole Genome Panel (2 multiplexes of 81 amplicons averaging 161 bps)

Hair Study



Tested 1 mm and 5 mm cuttings from 60 hair shafts (120 samples).

Approximately 1 cm of the root end was removed, followed by the 5 mm cutting, and finally the 1 mm cutting.



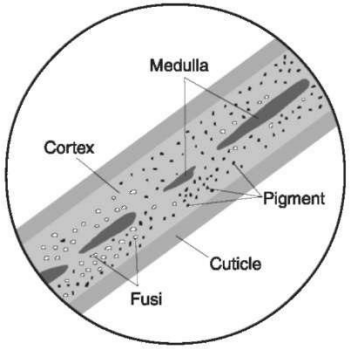
Hair Study

Head Hairs in Three Different Age Ranges:

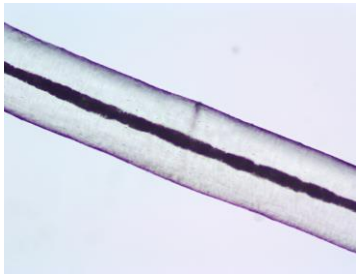
Recent (R) = <5 years of age (13 hairs)
Old (O) = 5-27 years, avg of 13.6 (24 hairs)
Older (VO) = 41-46 years, avg of 43.4 (23 hairs)

Provided by PSU, Mitotyping, and MicroTrace
IRB STUDY's 00000970 & 00014305

Microscopic Characterization



- Characterized on a Leica FS 4000 comparison microscope
- Medulla structure
- Diameter
- Other characteristics such as pigment, ovoid bodies, cortical fusi, cuticle structure, physical damage



Hair Extraction

LMB
1x Terg-a-zyme Wash
Qiagen ATL buffer
Proteinase K
DTT
Qiagen AL Buffer
Magnetic Beads
Isopropanol
PrepFiler Wash Buffer A
PrepFiler Wash Buffer B
PrepFiler Elution Buffer

LMB = **L**yse (dissolve) & **M**agnetic **B**eads

40 uL extract

Forensic Science International: Genetics 32 (2018) 7–17

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Research Paper

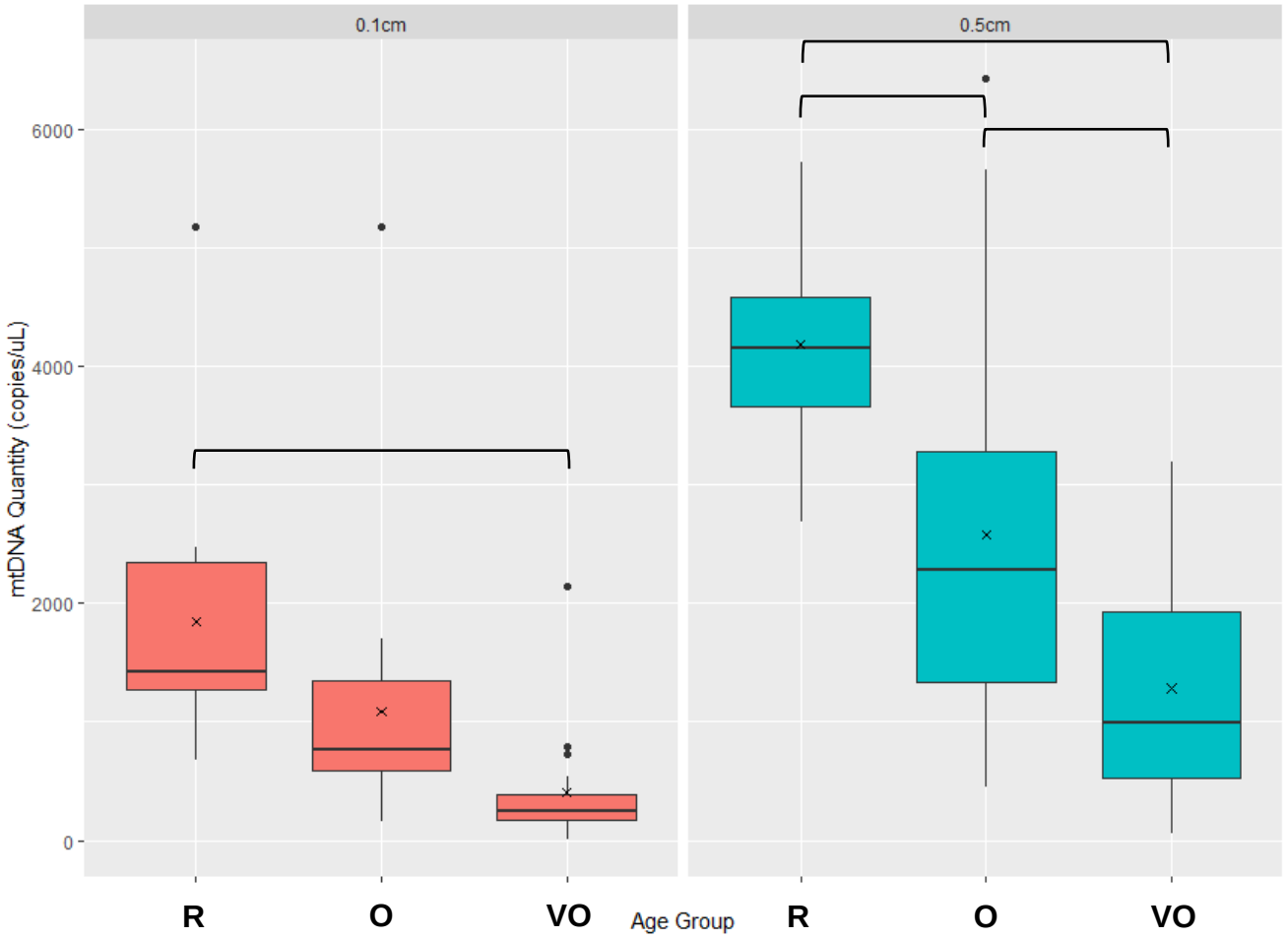
Assessing heteroplasmic variant drift in the mtDNA control region of human hairs using an MPS approach

Jamie M. Gallimore, Jennifer A. McElhoe, Mitchell M. Holland*

Forensic Science Program, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, 014 Thomas Building, University Park, PA 16802, United States

Custom mtqPCR assay to assess both quantification and degradation

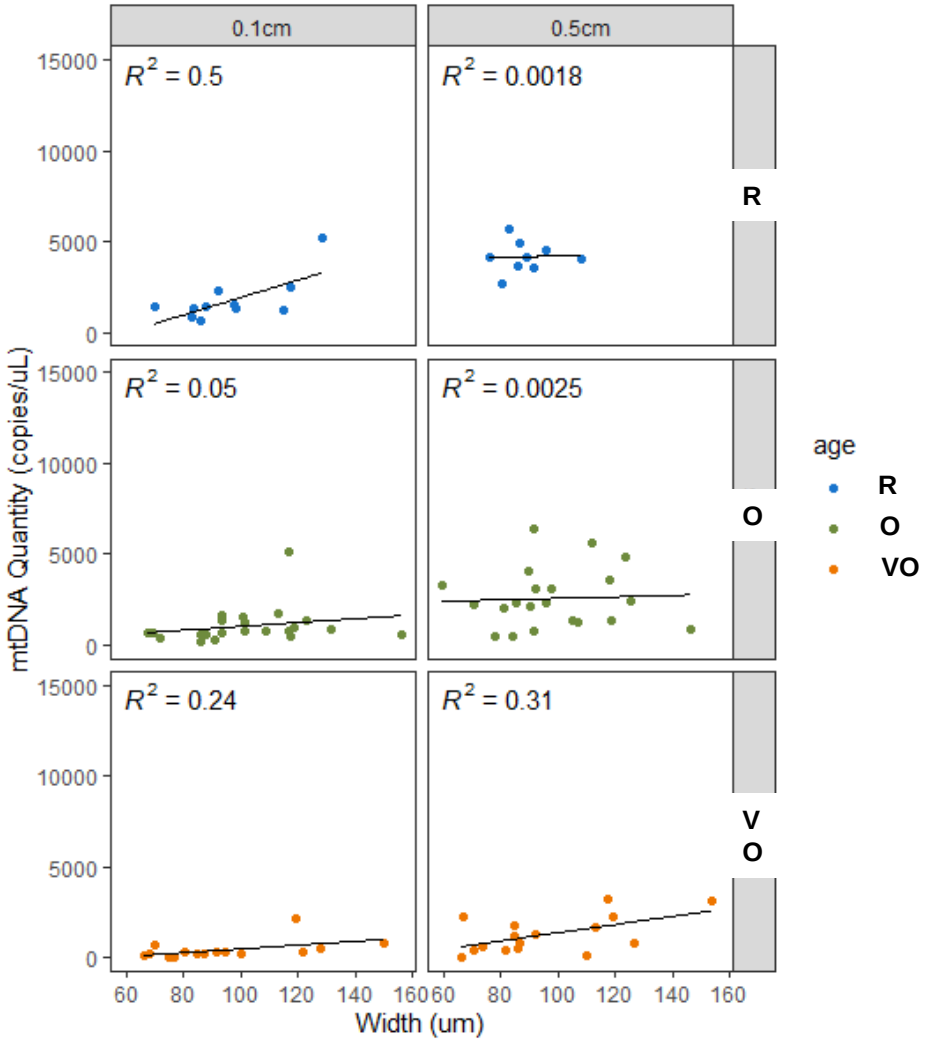
mtDNA Yield v. Age of the Hair



p-value < 0.05 (within)

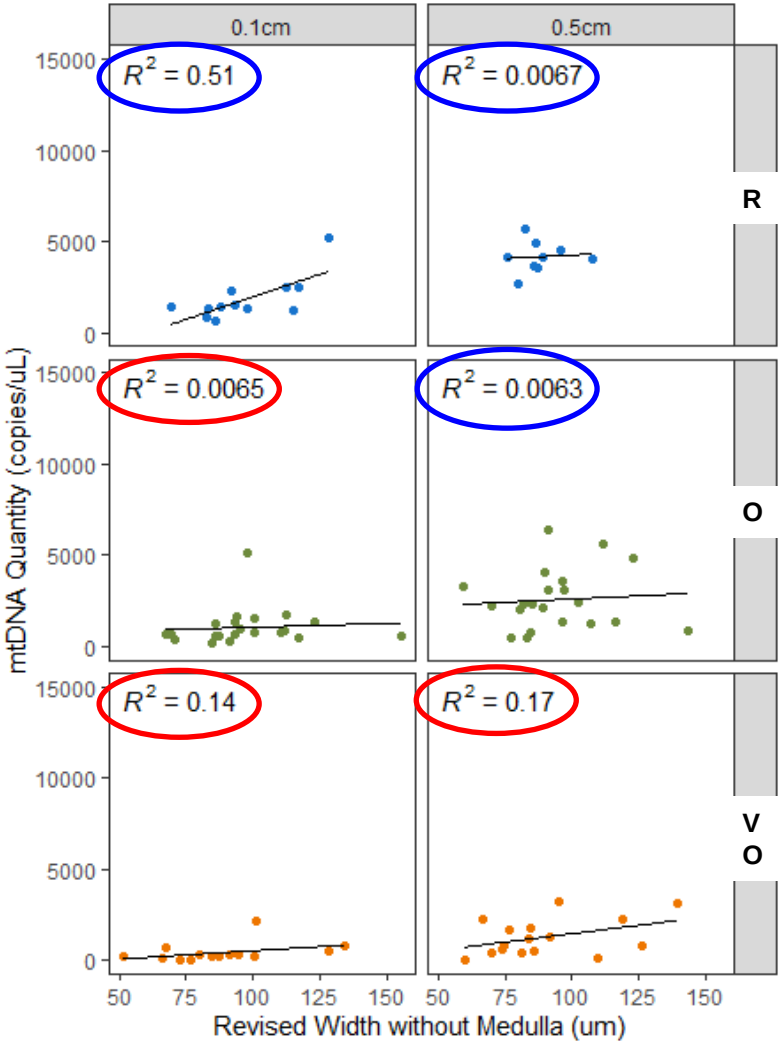
= median, * = mean

mtDNA Yield v. Width of Hair



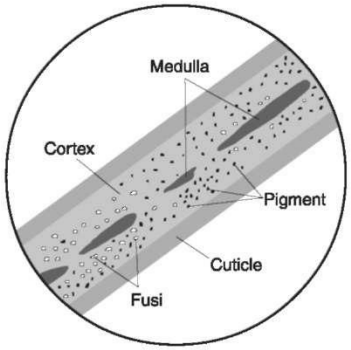
R = <5 yo
O = 13.6 yo
VO = 43.4 yo

mtDNA Yield v. Width of Hair



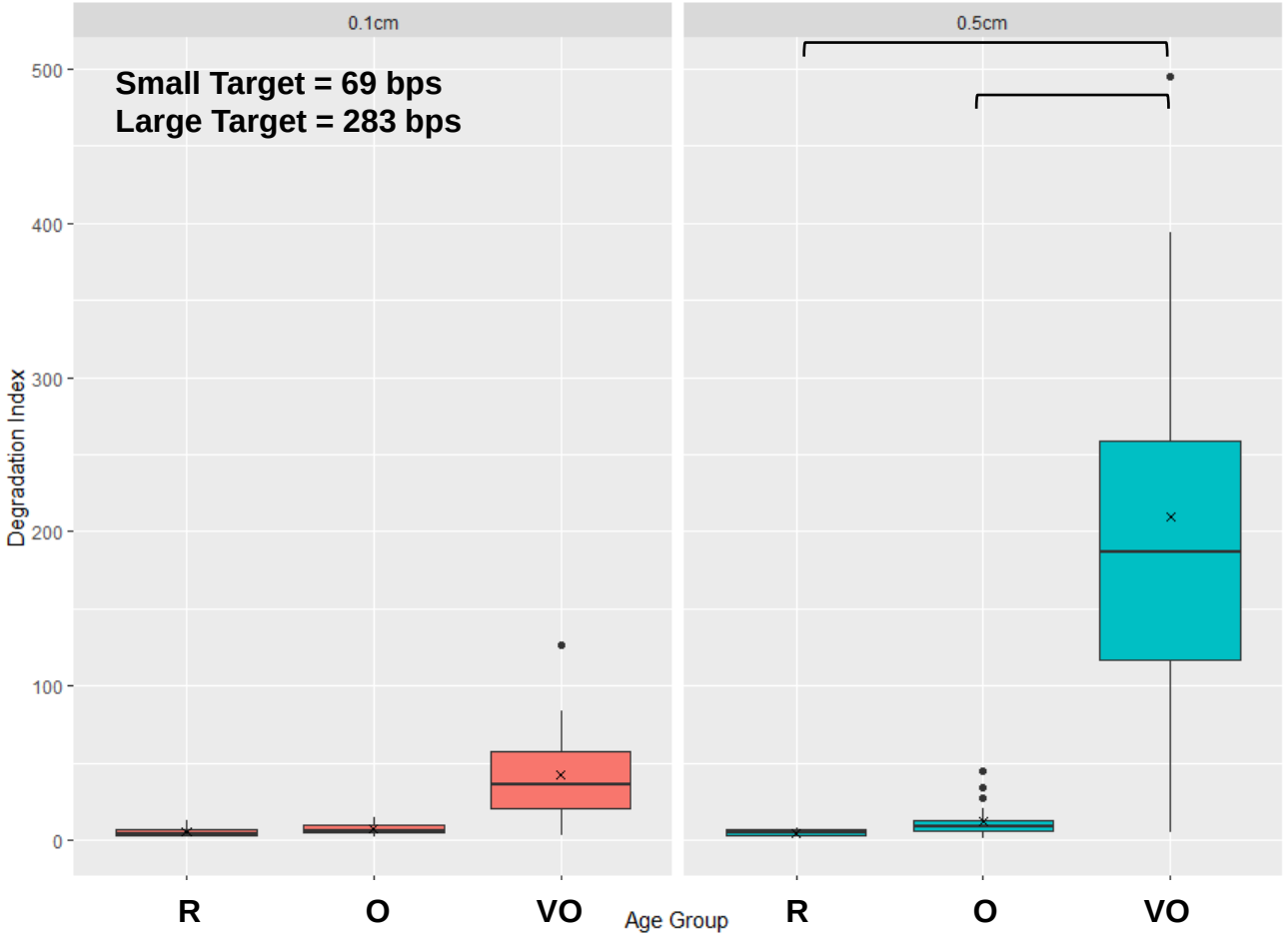
= Better

= Worse



Width without a medulla

Degradation Index (DI) v. Age



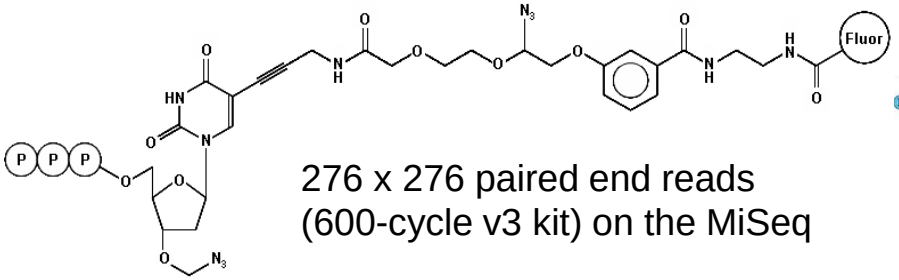
p-value < 0.05 (within)

R = <5 yo
O = 13.6 yo
VO = 43.4 yo

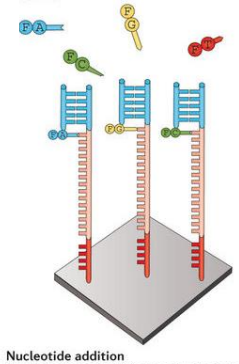
Reverse Terminator Sequencing

PowerSeq WGM (mitogenome, 1 multiplex of 161 amplicons averaging 167 bps)

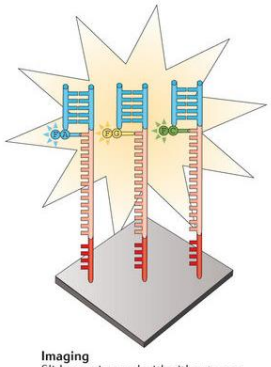
Sequencing on the Illumina MiSeq



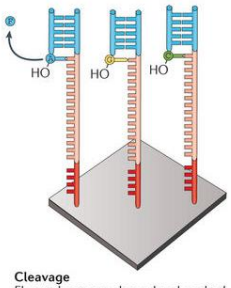
276 x 276 paired end reads
(600-cycle v3 kit) on the MiSeq



Nucleotide addition
Fluorophore-labelled, terminally blocked nucleotides hybridize to complementary base. Each cluster on a slide can incorporate a different base.



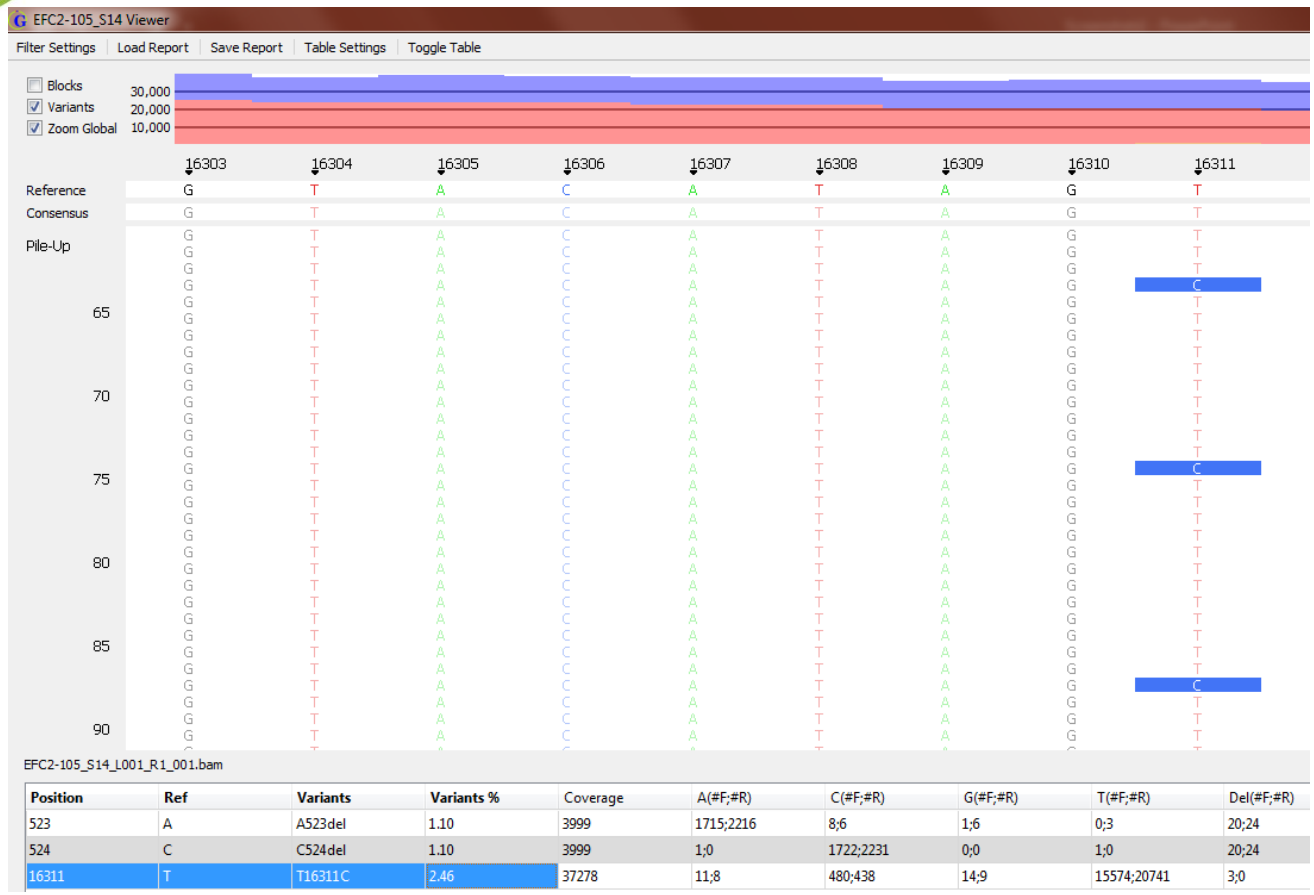
Imaging
Slides are imaged with either two or four laser channels. Each cluster emits a colour corresponding to the base incorporated during this cycle.



Cleavage
Fluorophores are cleaved and washed from flow cells and the 3′-OH group is regenerated. A new cycle begins with the addition of new nucleotides.

Goodwin et al., Nat Genet Review 2016

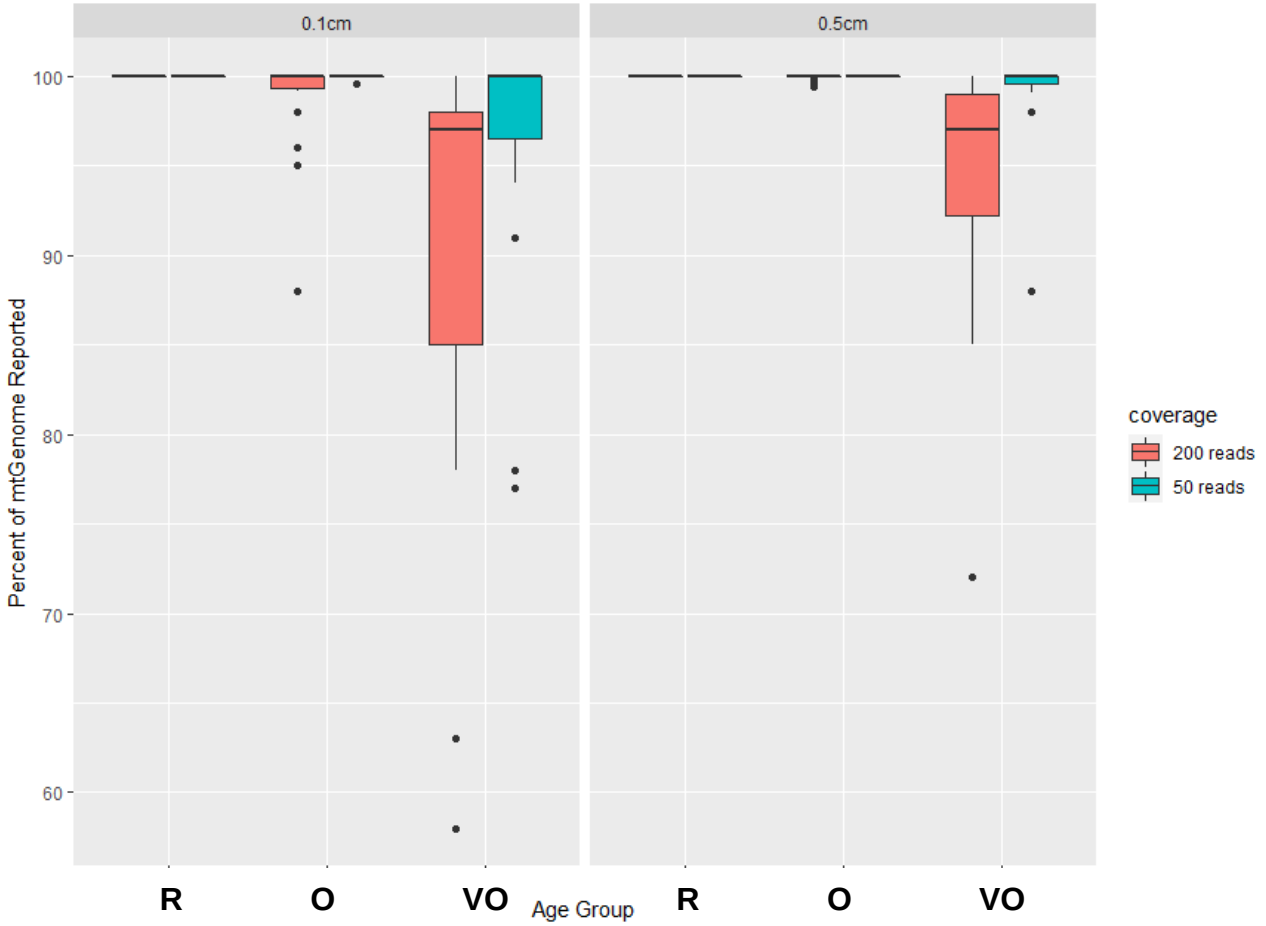
Data Analysis



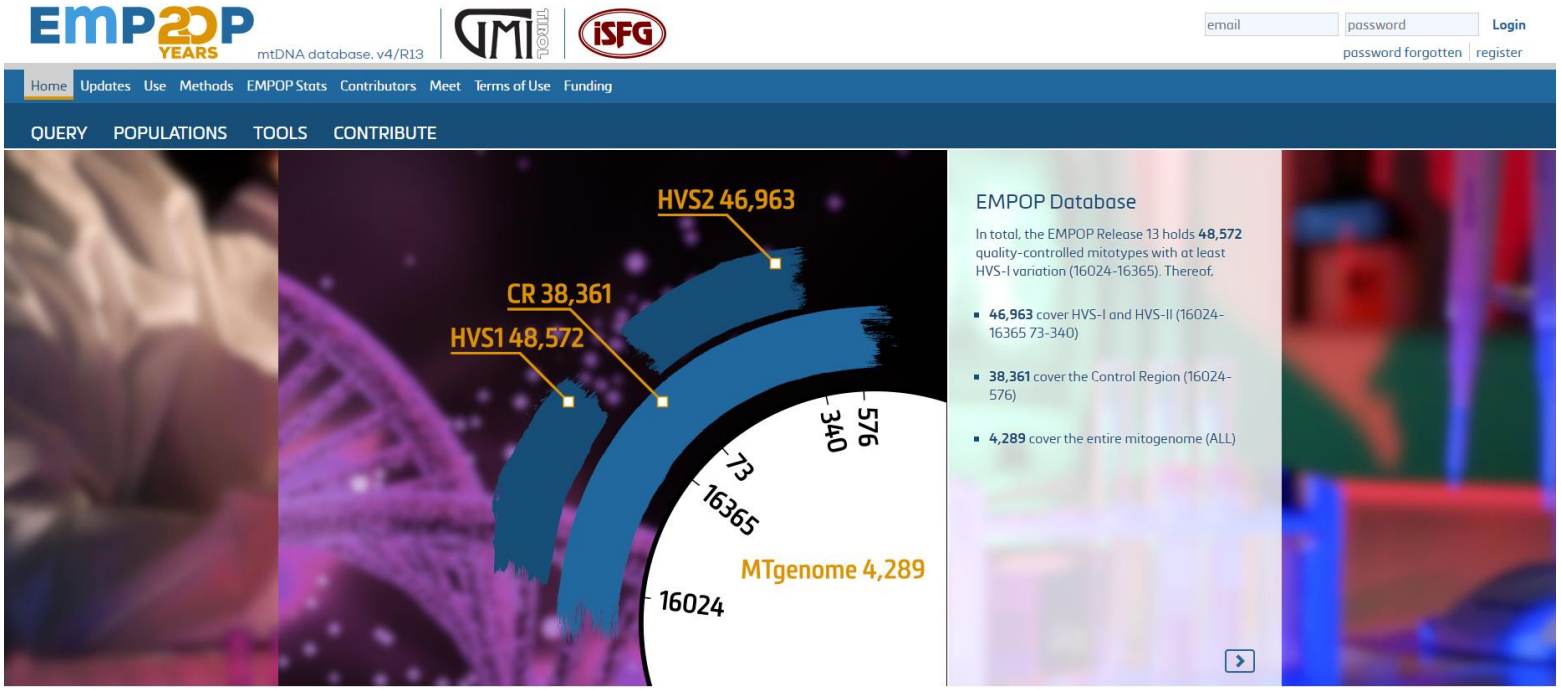
Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)
523	A	A523del	1.10	3999	1715;2216	8;6	1;6	0;3	20;24
524	C	C524del	1.10	3999	1;0	1722;2231	0;0	1;0	20;24
16311	T	T16311C	2.46	37278	11;8	480;438	14;9	15574;20741	3;0

Holland et al., FSIG 2017
GeneMarker™ HTS

Percent of the mitoGenome Reported v. Age



If mitogenome sequencing is to be adopted by the forensic community, a database of haplotypes will be required to provide weight estimates for a match



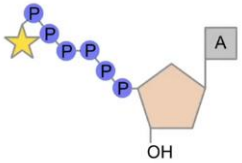
The 10,000 mitogenome project!

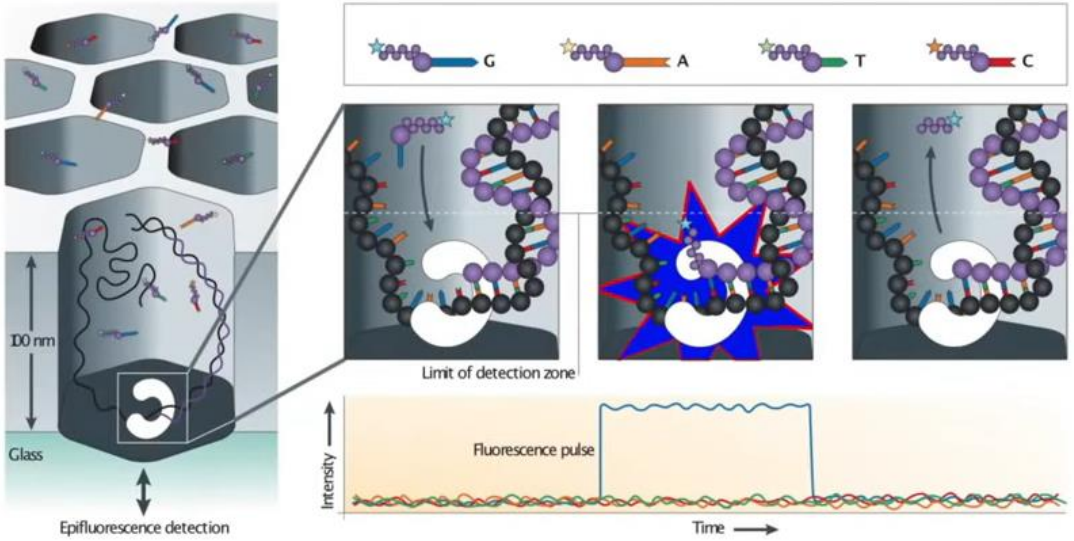
empop.online

PacBio Sequel IIe HiFi Sequencing

SMRTbell

Single Molecule Real Time

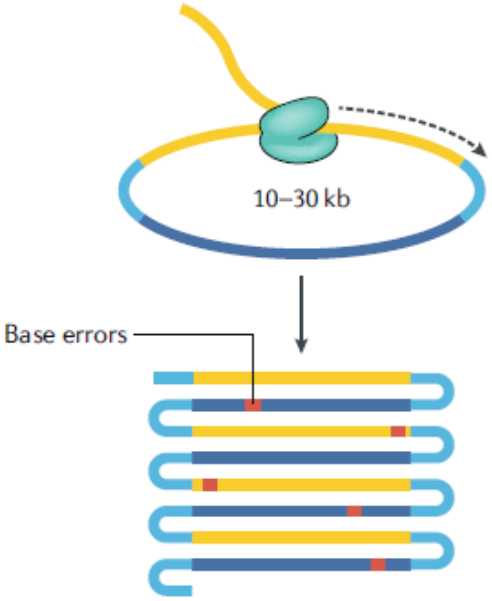


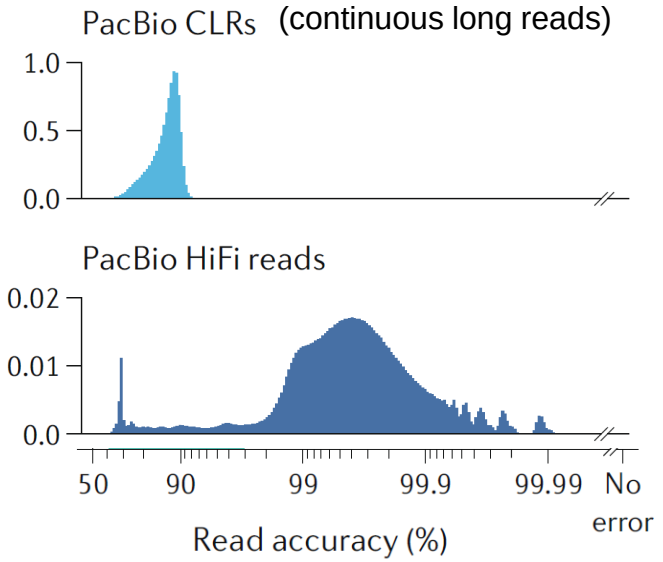


Nature Reviews Genetics **11**, 31–46 (2010)

Reaction volume is ~20 zeptoliters (10^{-21} liters). During a single incorporation, the fluorescent dye is detected and released.

PacBio HiFi Accuracy





The polymerase sequences the template 8+ times …

… significantly enhancing read accuracy; i.e., reducing error

Logsdon 2020, Nature Reviews

Extract liquid blood samples: 22-23 at one time, with 1-2 reagent blanks per set of 24 total samples

A

B

LR amplify 4 uL of extract. Two amps of **~8.5 kb each (A & B)** to cover the mitogenome, with unique sets of indices (barcodes) for each amp. Amplify 93 extracts + 2 RBs + 1 Neg x **two** plates

Combine **A & B amps** and normalize on a SequalPrep™ **Normalization** Plate

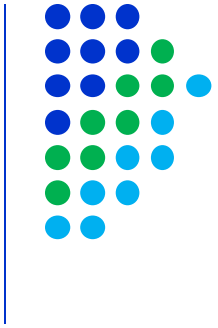
Concentrate the eluted products to a volume of **~150 uL** with a 100k microcon filter

Repeat the process **three times**, for a total of **372** blood samples, 8 RBs, and 4 Neg controls

Combine the four runs, concentrate again in a 100k microcon to a **final volume of ~150 uL**

Send to the core lab for **SMRTbell prep & sequencing** of 372 blood samples per run

At a cost of ~$23 per sample



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