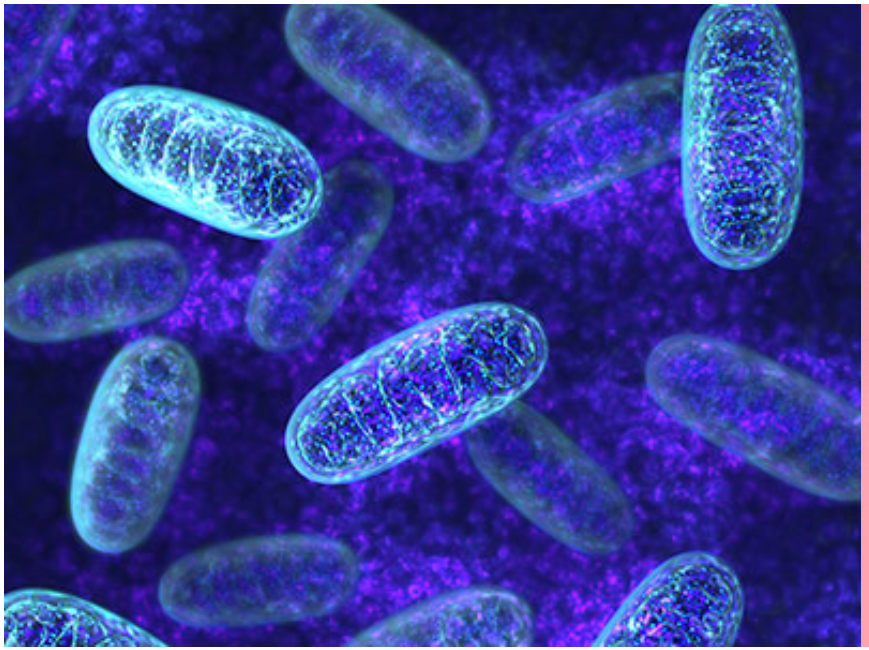
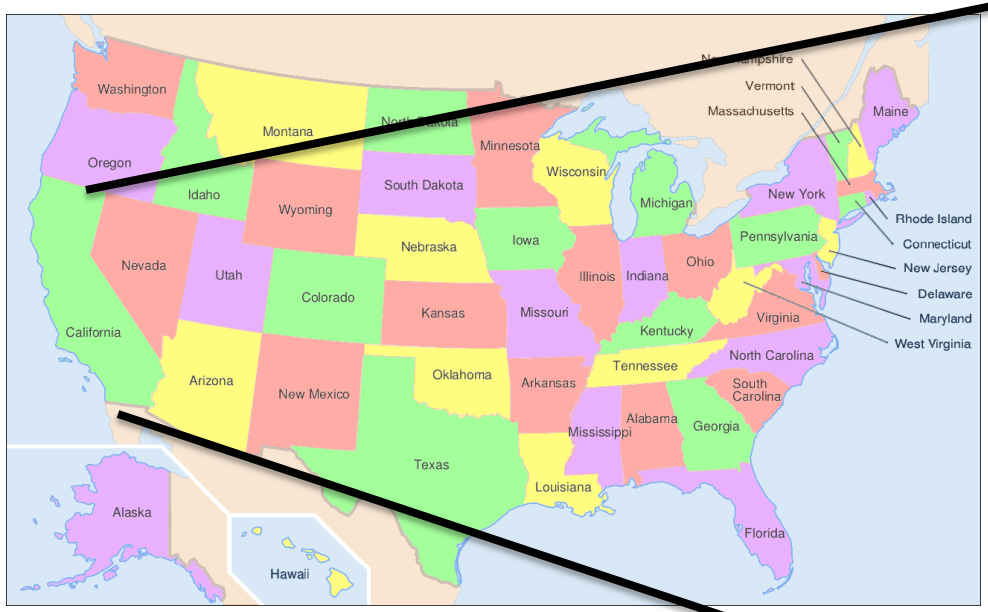
# Implementing an MPS mtGenome Panel into Casework in a Missing Persons DNA Program




California Department of Justice – Jan Bashinski DNA Laboratory
Daniela Cuenca, Criminalist

References: https://innovationdistrict.childrensnational.org/mitochondria-key-repairing-cell-damage-dmd/; http://thespringcenterfornaturalmedicine.com/immune-system/happy-mitochondria-happy-life/

# California Department of Justice Bureau of Forensic Services Laboratories



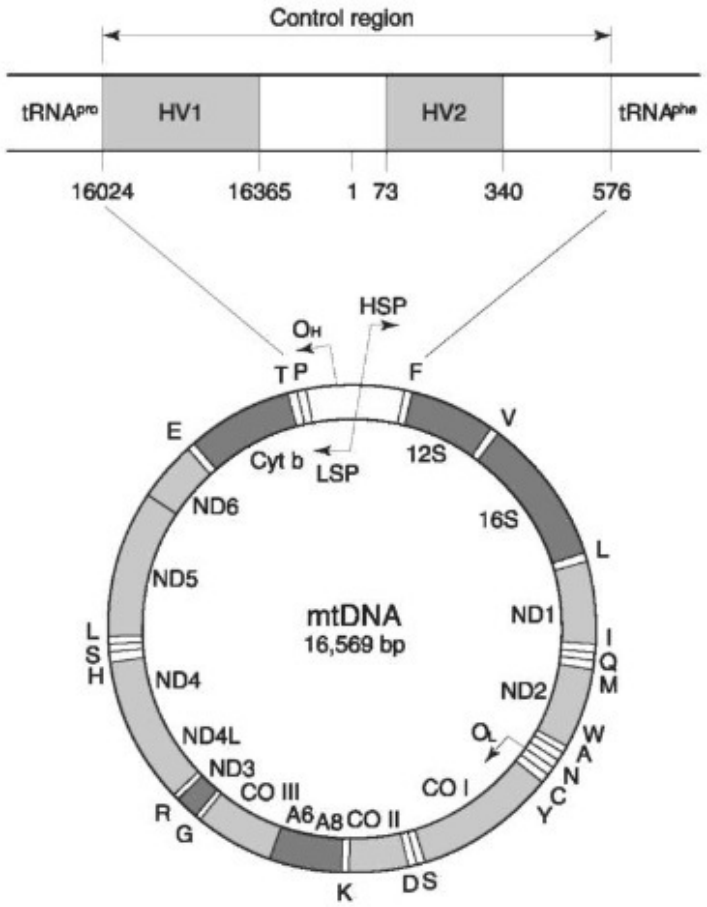


11 Laboratories

7 DNA Laboratories

# Roche Mitochondrial Duplex (Sanger Sequencing)

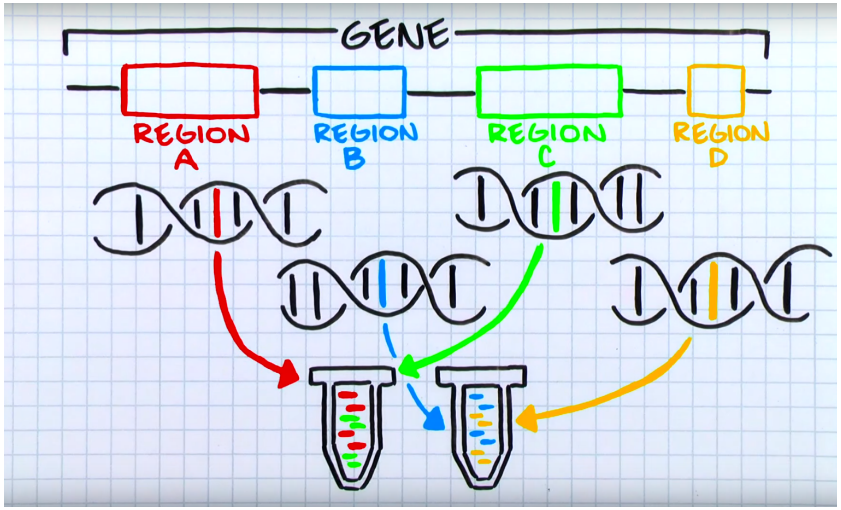
- Targets mitochondrial hypervariable region 1 and 2 in two amplicons
  - Used to establish lineage or/and when DNA is limited (e.g. hair shaft) or extremely degraded

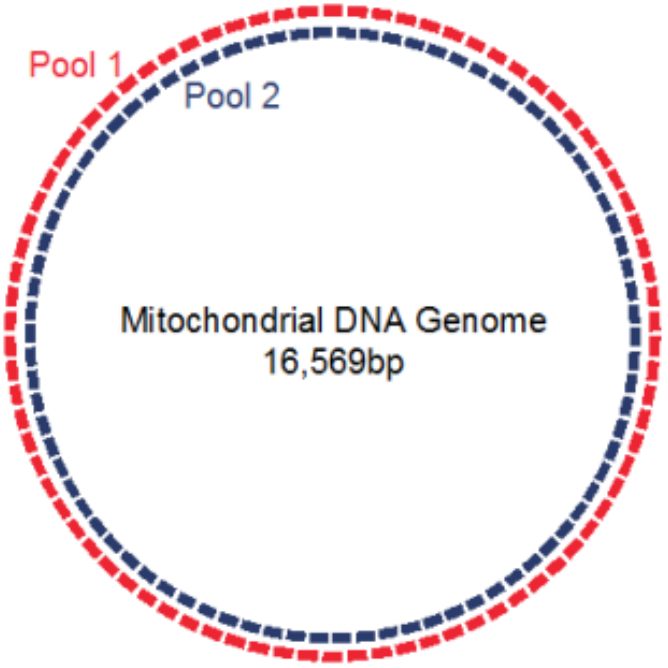



Reference: http://www.nfstc.org/pdi/Subject09/pdi_s09_m02_01_a.htm

# Applied Biosystems™ Precision ID Whole Genome Panel

- Thermo Fisher Scientific -Ion Torrent commercial kit
- AmpliSeq –library prep chemistry
- 162 amplicons in two PCR reactions (81 primer pairs per PCR Rxn)
- 163 bp average amplicon size
- >118 degenerate primers per PCR Rxn

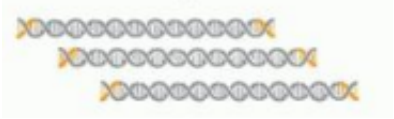



Reference: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0015831_PrecisionID_Panels_IonS5_UG.pdf; https://www.youtube.com/watch?v=D3Dv7QHpLcs

# Applied Biosystems™ Precision ID Whole Genome Panel Workflow

## Library Preparation


### PCR

- DNA amplification of the mitochondrial genome in 2 PCR reactions
- 100 pg total input in 15 µL
- 8 samples per library preparation run

### Digest & Repair

- Partially digest primer regions of the amplicons
- Repair the amplicon ends

### Adaptor Ligation

- Ligate the clonal amplification and sequencing adaptors
- The adaptors include a unique barcode (1-32) to allow multiplexing

### Pool

- Bead normalize the libraries
- Pool together at equal volumes
- Dilute the pooled library

Reference: https://www.thermofisher.com/order/catalog/product/4475345

# Applied Biosystems™ Precision ID Whole Genome Panel Workflow

## Library Preparation

- Library Preparation is Automated by the Ion Chef

Load samples to the library preparation plate

Load the Ion Chef

- 7 hours per library preparation run

Reference: https://www.thermofisher.com/order/catalog/product/4484177?ICID=search-product
http://www.brand.de/en/products/life-science-products/pcr-disposable-products/pcr-plates/

# Applied Biosystems™ Precision ID Whole Genome Panel Workflow

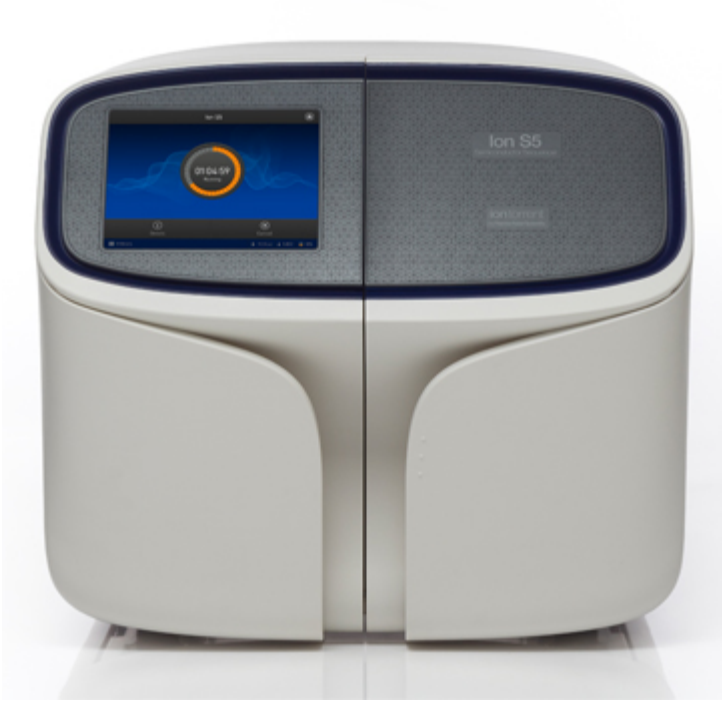
## Clonal Amplification and Sequencing

### Templating and Chip Loading

- Emulsion PCR / Clonal Amplification
- Loading Sequencing Chip
- Automated on the Ion Chef
- ~ 12 hours


### Sequencing

- ~ 2 hours for sequencing
- ~ 6 hours for signal processing

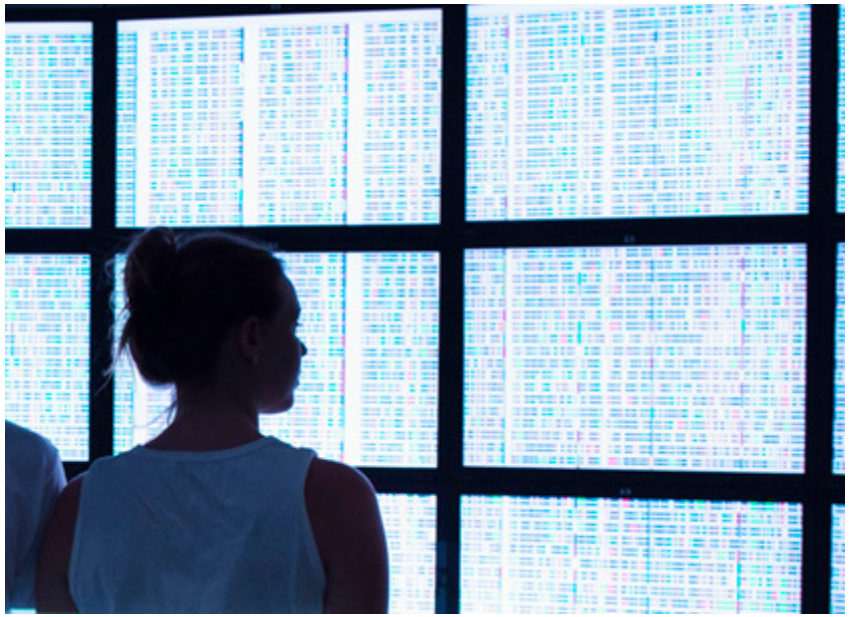



Reference: https://www.thermofisher.com/order/catalog/product/4484177?ICID=search-product
http://www.brand.de/en/products/life-science-products/pcr-disposable-products/pcr-plates/

# At a Glance

| | Precision ID Assay |
|---|---|
| **Library Prep Chemistry** | PCR – Adaptor Ligation |
| **Ideal DNA Input** | 100 pg |
| **Samples per Run** | 4-32 |
| **Sequencing Chemistry** | Semiconductor (ion) |
| **Sequencer** | Ion Torrent S5 |
| **Hands On Time** | <2 hours |
| **Full Time (extract to sequence)** | 48 hours (4 work days) |
| **Reads Per Run** | 9-14 Million |
| **Price (per sample*)-** | $ 201.79 (~€ 165) |

*Price per sample will vary depending on the amount of samples that are multiplexed together.



# Data

## Known Samples, Sensitivity and Analytical Threshold

Reference: https://news.ucsc.edu/2017/11/nih-data-commons.html

# The Power of The Whole Genome

Mitochondrial DNA resolution can increase from 64-76% with HVI and HVII sequencing to 98-100% with whole genome sequencing

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]

[a] Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[b] Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria
[c] Penn State Eberly College of Science, University Park, PA, USA
[d] Department of Forensic Medicine, Hjelt Institute, P.O. Box 40, 00014 University of Helsinki, Helsinki, Finland
[e] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

Forensic Science International: Genetics 10 (2014) 73–79

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

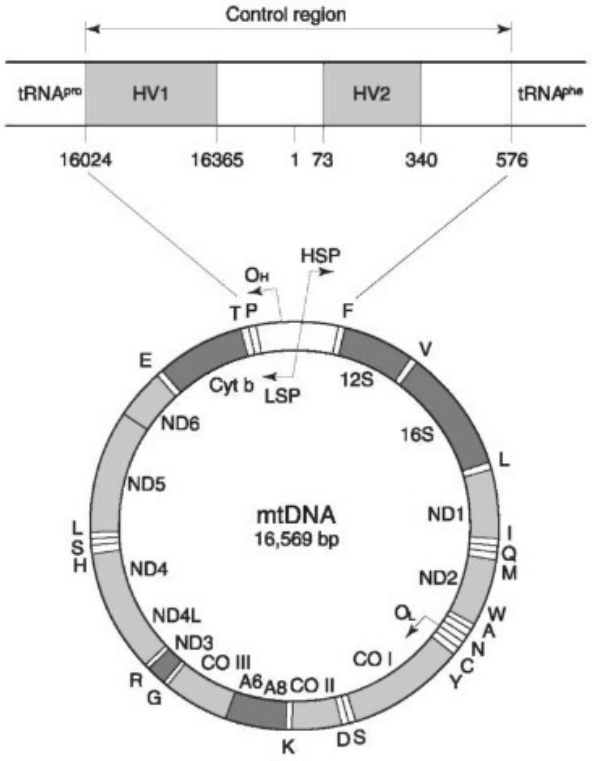
Short Communication

## Development of forensic-quality full mtGenome haplotypes: Success rates with low template specimens

Rebecca S. Just [a,b,c,*], Melissa K. Scheible [a,b], Spence A. Fast [a,b], Kimberly Sturk-Andreaggi [a,b], Jennifer L. Higginbotham [a,b], Elizabeth A. Lyons [a,b,1], Jocelyn M. Bush [a,b], Michelle A. Peck [a,b], Joseph D. Ring [a,b], Toni M. Diegoli [a,b], Alexander W. Röck [d], Gabriela E. Huber [d], Simone Nagl [d], Christina Strobl [d], Bettina Zimmermann [d], Walther Parson [d,e], Jodi A. Irwin [a,b,2]

[a] Armed Forces DNA Identification Laboratory, 115 Purple Heart Dr., Dover AFB, DE 19902, United States
[b] American Registry of Pathology, 120A Old Camden Rd., Camden, DE 19934, United States
[c] University of Maryland, College Park, 8082 Baltimore Ave., College Park, MD 20740, United States
[d] Institute of Legal Medicine, Innsbruck Medical University, Müllerstrasse 44, Innsbruck, Austria
[e] Penn State Eberly College of Science, 517 Thomas Building, University Park, PA 16802, United States

# The Power of The Whole Genome




HVI/HVII Current Method

(Roche Mitochondrial Duplex Assay)

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| HVII | 263G | 263G | 263G |
| | 315.1C | 315.1C | 315.1C |
| HVI | ---------- | ---------- | ---------- |

Samples 1-3 are indistinguishable from each other when using current methods.

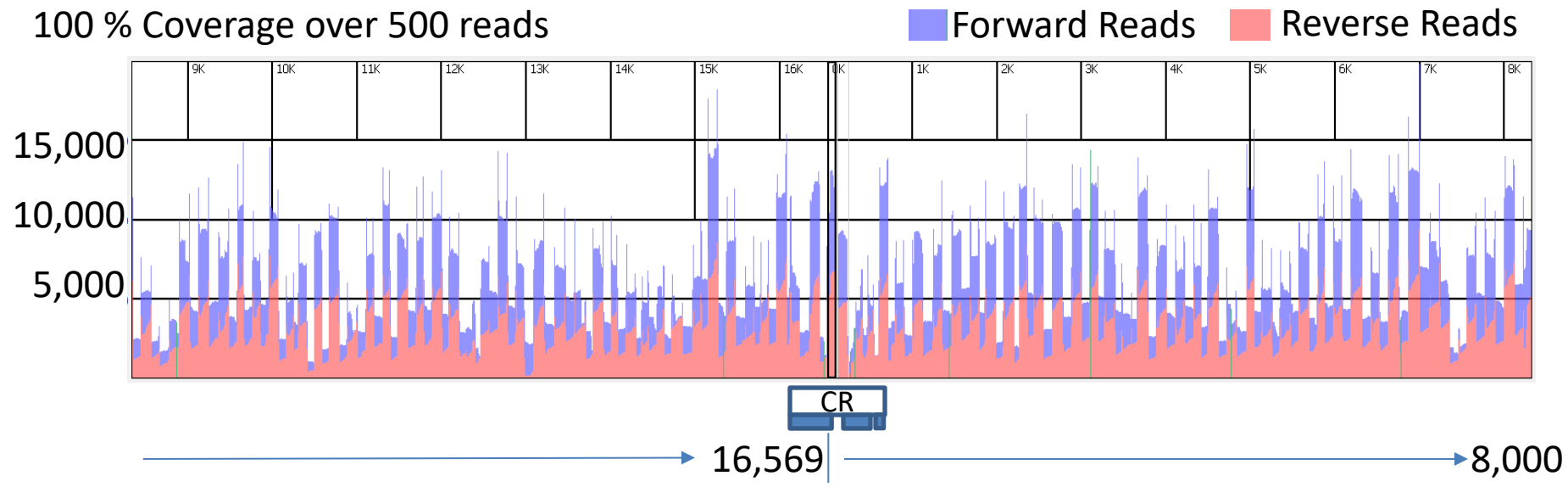
Reference: http://www.nfstc.org/pdi/Subject09/pdi_s09_m02_01_a.htm

# H3, H1, H4

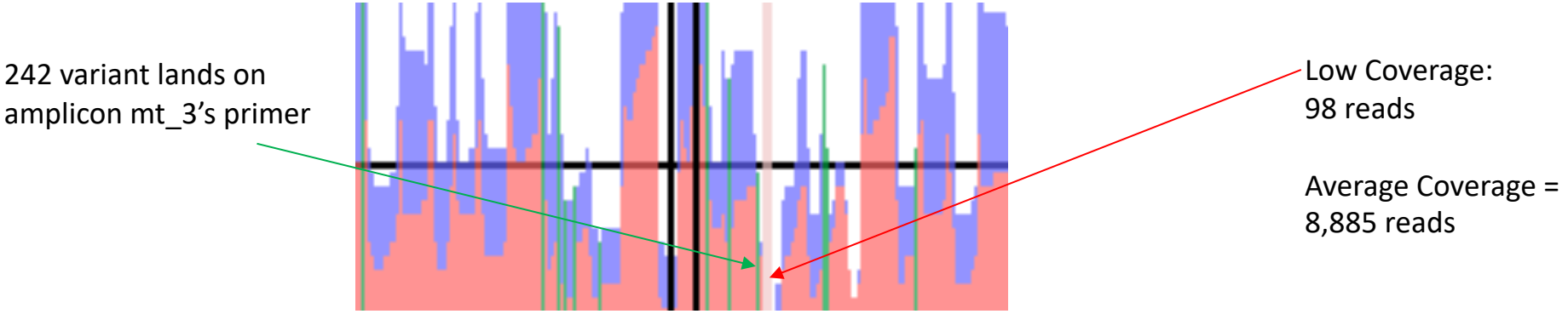
| Sample 1 | Sample 2 | Sample 3 |
|---|---|---|
| 263G | 263G | 263G |
| 315.1 | 315.1 | 315.1 |
| | 477C | |
| 750G | 750G | 750G |
| 1018A | | |
| 1438G | 1438G | 1438G |
| | 3010A | |
| | | 3992T |
| | | 4024G |
| 4769G | 4769G | 4769G |
| | | 5004C |
| 6776C | | |
| | | 8269A |
| 8860G | 8860G | 8860G |
| | | 9123A |
| | | 10044G |
| | 14350T | |
| | | 14365T |
| | | 14582G |
| 15326G | 15326G | 15326G |
| 16519C | 16519C | |

# Applied Biosystems™ Precision ID Whole Genome Panel

## Reference Samples




Variants positioned in a primer region will cause a mismatch that will lead to a low performing amplicon. Reason for the many degenerate primers added to improve the assay.



# Applied Biosystems™ Precision ID Whole Genome Panel

## Sensitivity

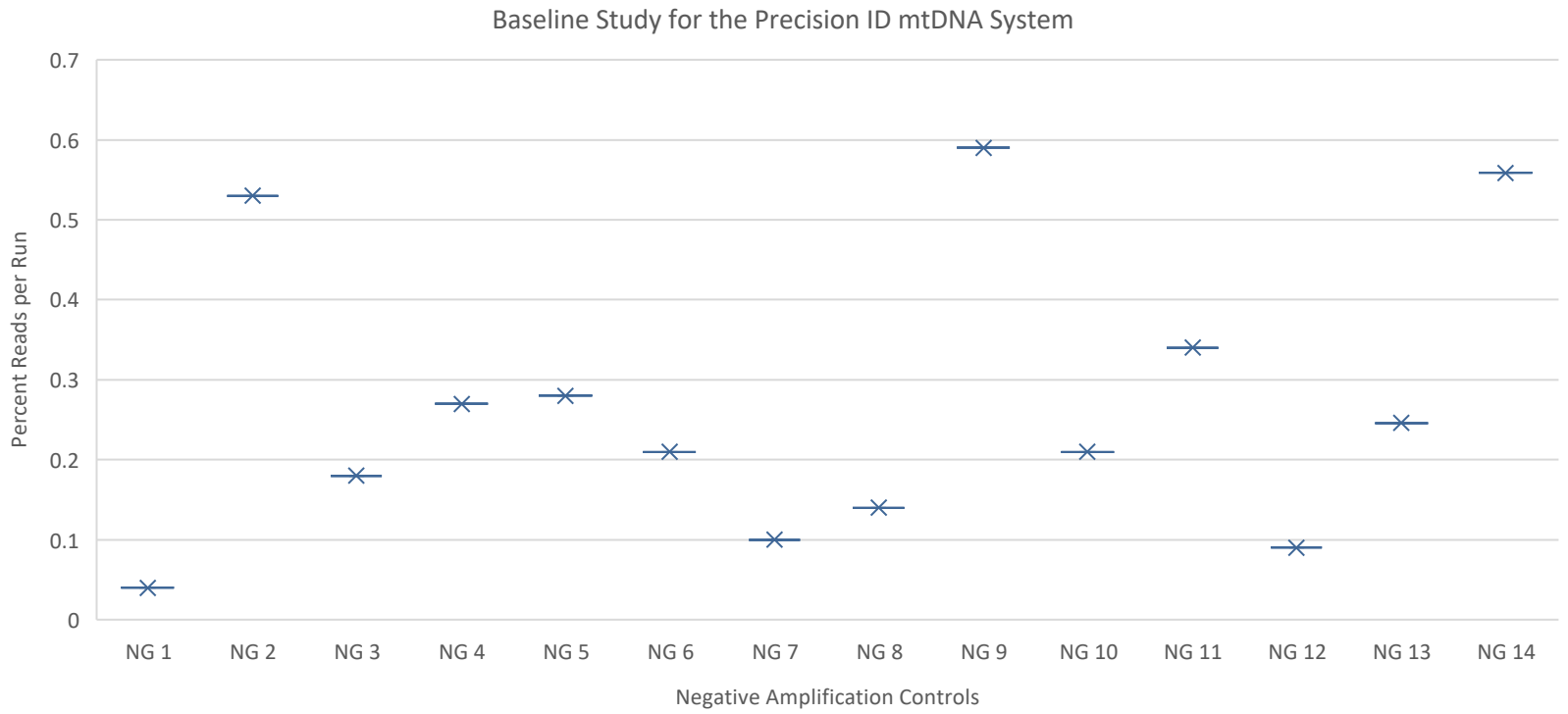
| Sample Description | Mito Copies | 1000 X Coverage | Noise | Noise StDEV | Avg. Noise Reads |
|---|---|---|---|---|---|
| HL60- 500 pg | 227,065 | 100% | < 1 % | | |
| HL60- 100 pg | 45,413 | 100% | < 1 % | | |
| HL60- 50 pg | 22,707 | 100% | < 1 % | | |
| HL60- 10 pg | 4,541 | 100% | 1% | 1.3% | 52 |
| HL60- 5 pg | 2,271 | 100% | 2.4% | 3.3% | 185 |
| HL60- 2.5 pg | 1,135 | 94.1% | 5.9% | 5.8% | 129 |
| HL60- 1 pg | 454 | 71.5% | 7% | 9.4% | 111 |

The first sign of low level template effects is observed at 10 pg
Signal Noise starts to appear above 10% at 2.5 pg

The 1 pg sample is not interpretable with a 10% threshold

# Applied Biosystems™ Precision ID Whole Genome Panel

## Noise Evaluation and Analytical Threshold




| Avg. | SD | Avg. + (SD X 3) LOD | Avg. + (SD X 10) LOQ | MIN | MAX | MAX + (SD X 10) |
|---|---|---|---|---|---|---|
| 0.27 % | 0.17% | 0.78% | 1.97% | 0.04% | 0.59% | 2.23% |

| | |
|---|---|
| Analytical Threshold for 8 samples | 2.5 % |
| Analytical Threshold for 16 samples | 1.25 % |
| Analytical Threshold for 24 samples | 0.83% |
| Analytical Threshold for 32 samples | 0.63% |

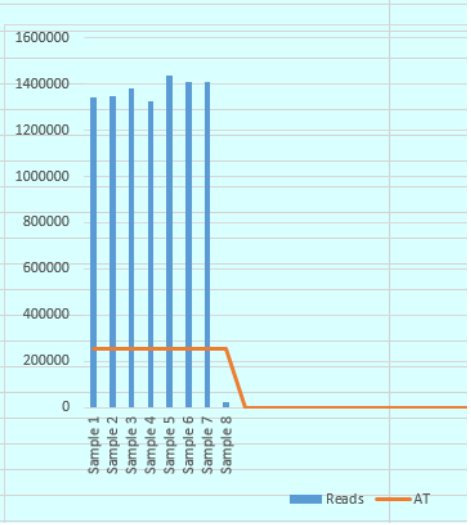
# Applied Biosystems™ Precision ID Whole Genome Panel

## Analytical Threshold

**Precision ID mtDNA Genome Analytical Threshold Workbook**

| Analytical Treshold Used : | 2.5% |
|---|---|
| Total Run Reads: | 10,120,643 |

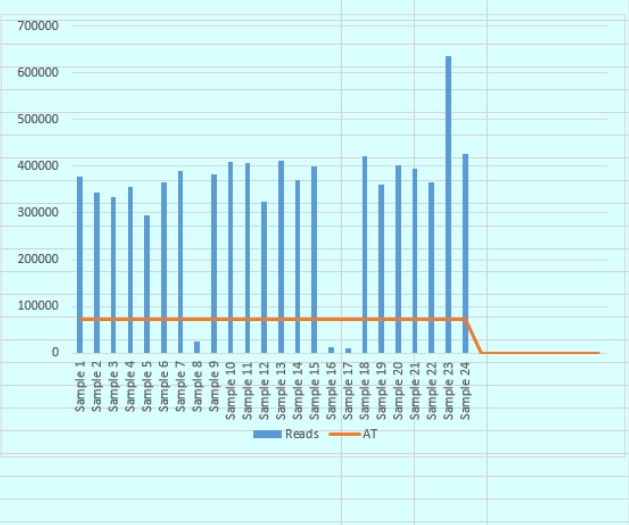
| | IonCode | Sample Name | Reads |
|---|---|---|---|
| 1 | IonCode 0109 | Sample 1 | 1342420 |
| 2 | IonCode 0110 | Sample 2 | 1346884 |
| 3 | IonCode 0111 | Sample 3 | 1379973 |
| 4 | IonCode 0112 | Sample 4 | 1323218 |
| 5 | IonCode 0113 | Sample 5 | 1437382 |
| 6 | IonCode 0114 | Sample 6 | 1406983 |
| 7 | IonCode 0115 | Sample 7 | 1411544 |
| 8 | IonCode 0116 | Sample 8 | 21635 |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |
| 15 | | | |
| 16 | | | |



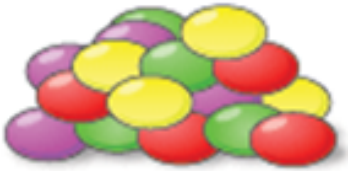

**Precision ID mtDNA Genome Analytical Threshold Workbook**

| Analytical Treshold Used : | 0.83% |
|---|---|
| Total Run Reads: | 8,596,289 |

| | IonCode | Sample Name | Reads |
|---|---|---|---|
| 1 | IonCode 0101 | Sample 1 | 378437 |
| 2 | IonCode 0102 | Sample 2 | 343516 |
| 3 | IonCode 0103 | Sample 3 | 333012 |
| 4 | IonCode 0104 | Sample 4 | 355559 |
| 5 | IonCode 0105 | Sample 5 | 295429 |
| 6 | IonCode 0106 | Sample 6 | 365384 |
| 7 | IonCode 0107 | Sample 7 | 390831 |
| 8 | IonCode 0108 | Sample 8 | 24312 |
| 9 | IonCode 0109 | Sample 9 | 382900 |
| 10 | IonCode 0110 | Sample 10 | 410191 |
| 11 | IonCode 0111 | Sample 11 | 406827 |
| 12 | IonCode 0112 | Sample 12 | 323754 |
| 13 | IonCode 0113 | Sample 13 | 412278 |
| 14 | IonCode 0114 | Sample 14 | 370918 |
| 15 | IonCode 0115 | Sample 15 | 400430 |
| 16 | IonCode 0116 | Sample 16 | 12162 |
| 17 | IonCode 0117 | Sample 17 | 9227 |
| 18 | IonCode 0118 | Sample 18 | 422355 |
| 19 | IonCode 0119 | Sample 19 | 361688 |
| 20 | IonCode 0120 | Sample 20 | 402530 |
| 21 | IonCode 0121 | Sample 21 | 395568 |
| 22 | IonCode 0122 | Sample 22 | 366469 |
| 23 | IonCode 0123 | Sample 23 | 635314 |
| 24 | IonCode 0124 | Sample 24 | 425822 |



Separated



Mixture

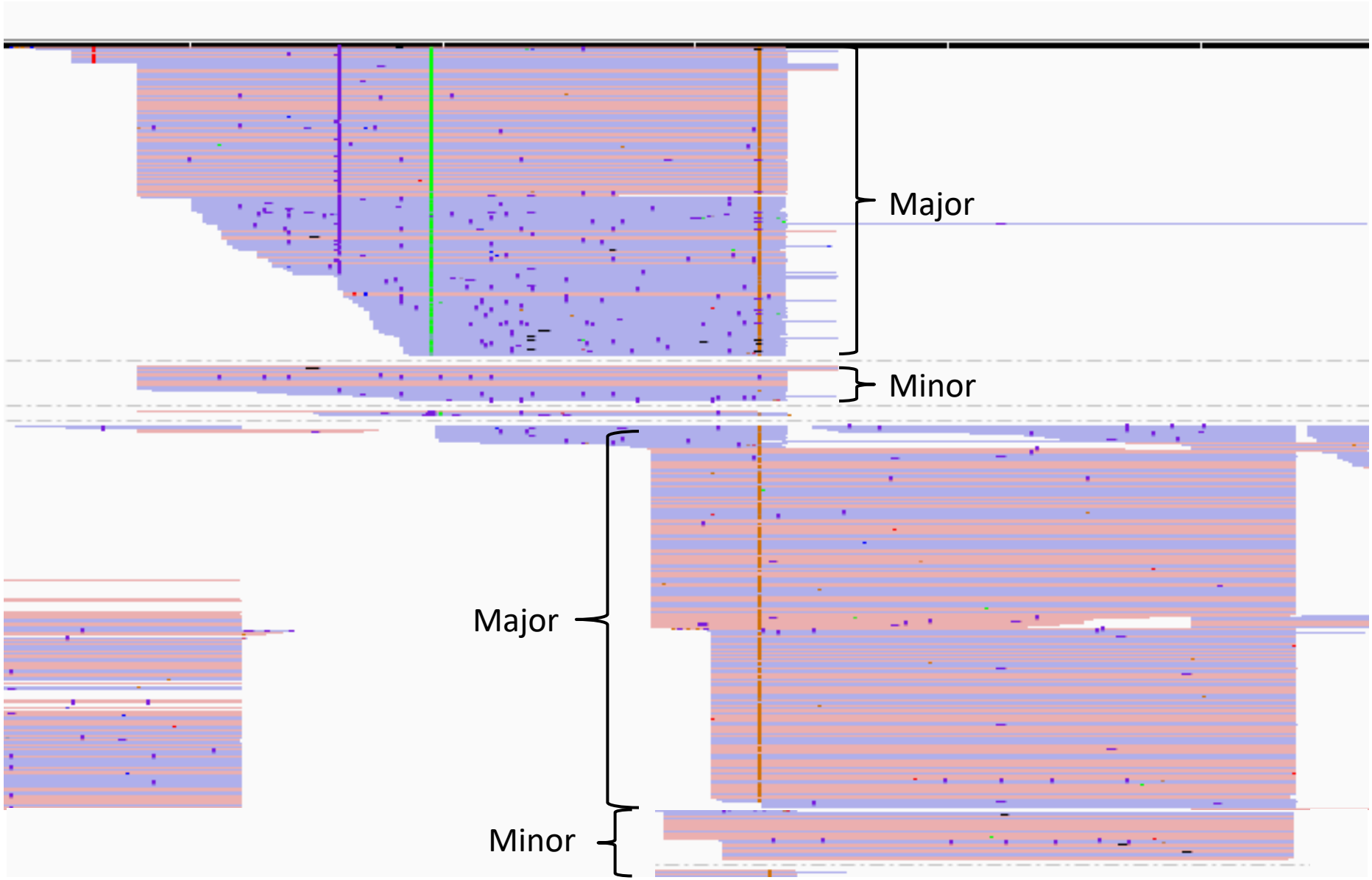
# Mixtures

## Uncharted Territory

Reference: http://www.bbc.co.uk/bitesize/ks3/science/chemical_material_behaviour/compounds_mixtures/revision/7/

# Applied Biosystems™ Precision ID Whole Genome Panel

## Mixtures



# Applied Biosystems™ Precision ID Whole Genome Panel

## Mixtures

### 2 person 1:1

| Position | P 1 | P 2 | P 1 % | P 2 % | Coverage |
|---|---|---|---|---|---|
| 73 | G | | 100 | | 15227 |
| 150 | T | C | 46.52 | 53.48 | 15598 |
| 152 | C | T | 46.62 | 53.38 | 15598 |
| 249 | del | A | 46.64 | 53.36 | 11242 |

### 2 person 2:1

| Position | P 1 | P 2 | P 1 % | P 2 % | Coverage |
|---|---|---|---|---|---|
| 73 | G | | 100 | | 11745 |
| 150 | T | C | 65.73 | 34.27 | 17185 |
| 152 | C | T | 65.95 | 34.05 | 17185 |
| 249 | del | A | 64.54 | 35.46 | 12231 |

### 2 person 9:1

| Position | P 1 | P 2 | P 1 % | P 2 % | Coverage |
|---|---|---|---|---|---|
| 73 | G | | 100 | | 10865 |
| 150 | T | C | 86.97 | 13.03 | 16019 |
| 152 | C | T | 87.15 | 12.85 | 16019 |
| 249 | del | A | 87.17 | 12.83 | 11696 |

### 2 person 19:1

| Position | P 1 | P 2 | P 1 % | P 2 % | Coverage |
|---|---|---|---|---|---|
| 73 | G | | 100 | | 8789 |
| 150 | T | C | 93.56 | 6.44 | 27848 |
| 152 | C | T | 93.7 | 6.3 | 27848 |
| 249 | del | A | 93.48 | 6.52 | 20285 |

### 3 person 1:1:1

| Position | Reference | Variant | Frequency | Coverage |
|---|---|---|---|---|
| 249 | A | del | 34.57 | 12048 |
| 250 | T | C | 35.4 | 5263 |
| 263 | A | G | 100 | 5333 |
| 452 | del | T | 31.2 | 8680 |
| 709 | G | A | 37.3 | 47580 |

### 3 person 1:3:5

| Position | Reference | Variant | Frequency | Coverage |
|---|---|---|---|---|
| 249 | A | del | 10.55 | 16441 |
| 250 | T | C | 33.7 | 6810 |
| 263 | A | G | 100 | 6953 |
| 452 | del | T | 32.8 | 11008 |
| 709 | G | A | 56.1 | 61392 |


# Degraded, Limited or Both!

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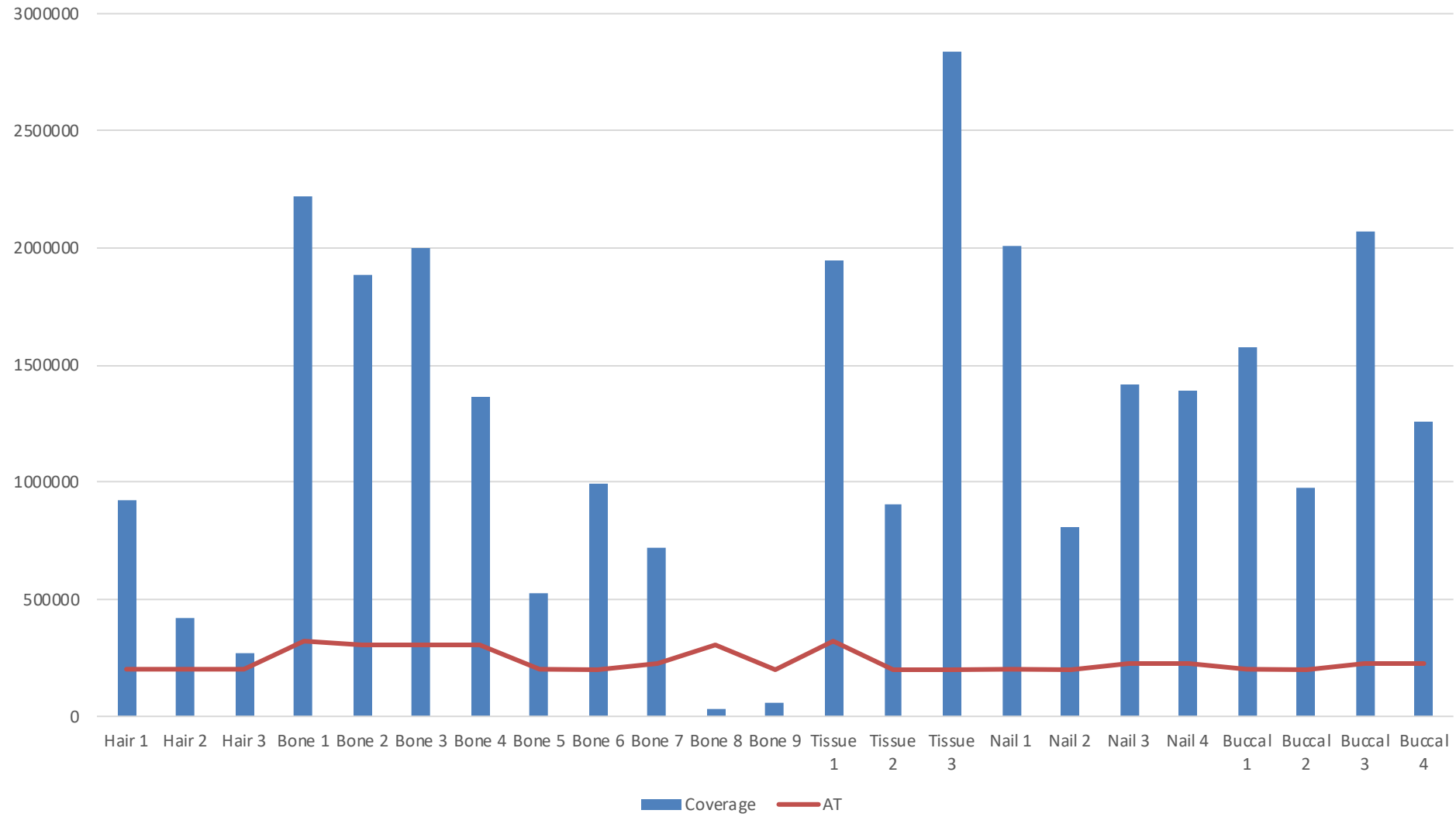
# Non-Probative Missing Persons DNA Samples

Reference: https://westminster.impacthub.net/2014/04/09/whats-the-biggest-challenge-preventing-you-from-delivering-your-social-vision/

# Applied Biosystems™ Precision ID Whole Genome Panel

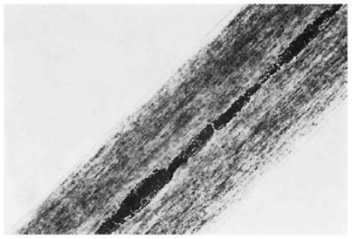
## Hair and Non-Probative Missing Persons DNA Samples




Total of 22 Samples

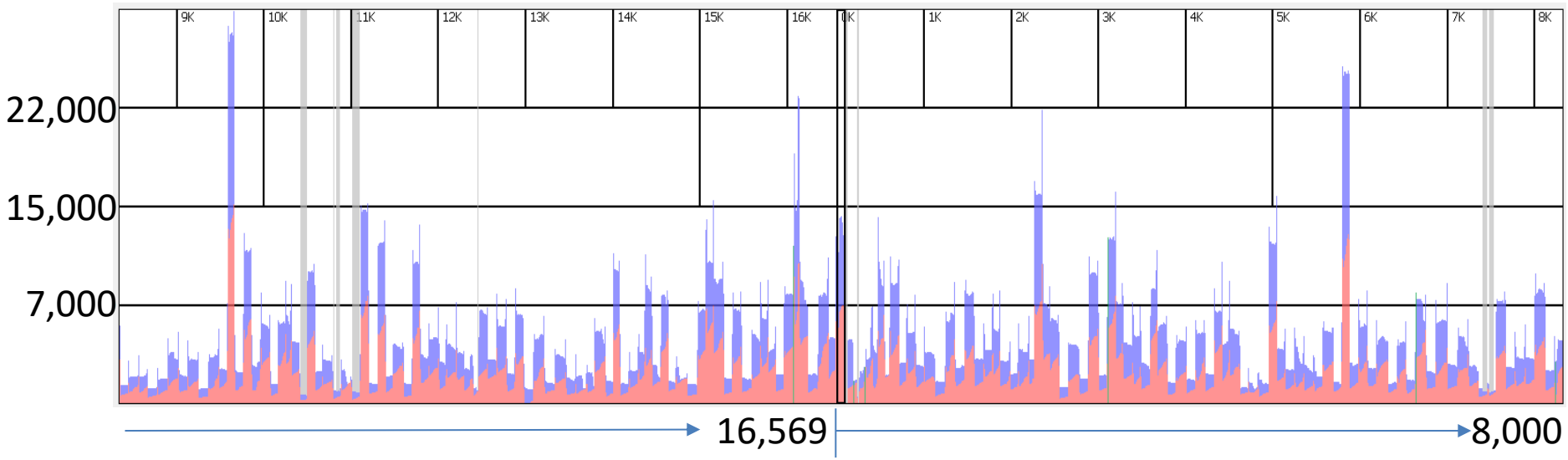
# Applied Biosystems™ Precision ID Whole Genome Panel

## Hair Shaft



Hair Sample- No STR Sanger results showed mixture

MPS results showed mixed bases at 5% (below threshold).




97.5% of Mitochondrial Genome

Reference: http://lh3.ggpht.com/_1wtadqGaaPs/TFGbwVFoJyI/AAAAAAAAMX4/3uNFx785voA/s1600-h/tmpE24_thumb3.jpg

# Applied Biosystems™ Precision ID Whole Genome Panel

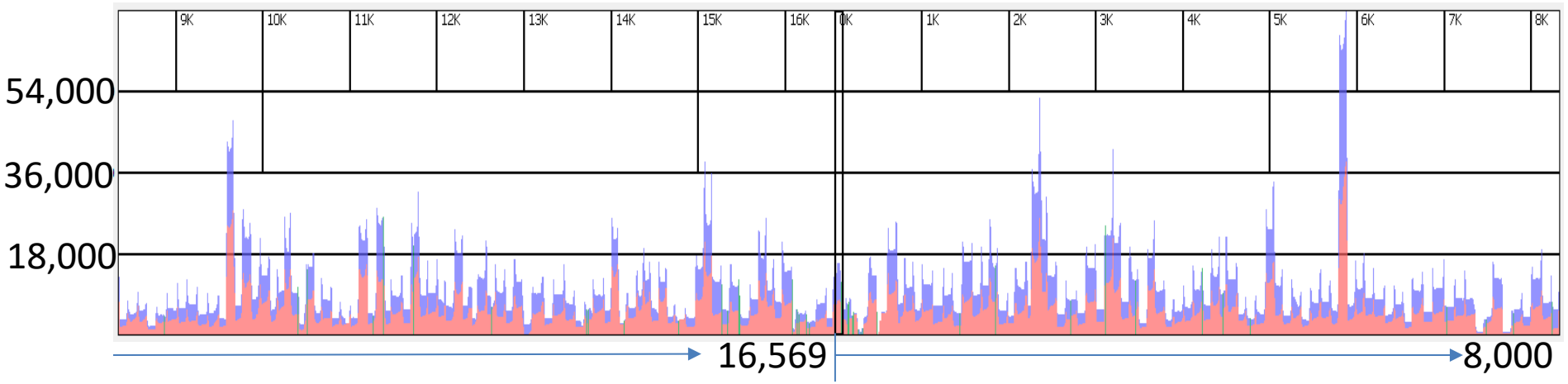
## Degraded Samples

Nuclear- Full With Minifiler

mtDNA- HVI and HVII (outsourced for small amplicons)

Trio Quant- 0.5 pg/µL nu large 10.6 pg/µL small DI: 21.2

Mito Quant- 6,550 mt copies/µL




Reference: https://www.getbodysmart.com/lower-limb-bones/femur-bone-anterior-markings

# Applied Biosystems™ Precision ID Whole Genome Panel

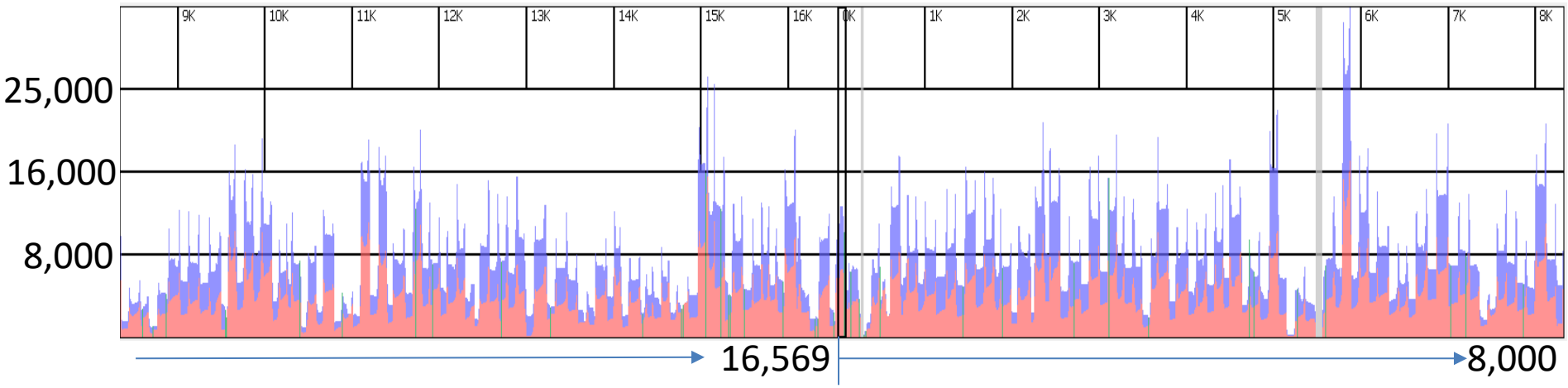
## Limited Samples

Nuclear- No STR

mtDNA- HVI and HVII (outsourced for small amplicons)

Trio Quant- 0.1 pg/µL nu large 0.2 pg/µL small DI: 2

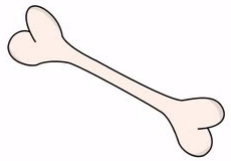
Mito Quant- 670 mt copies/µL




Reference: https://www.getbodysmart.com/skull-bones-review/skull-bone-markings-anterior-view

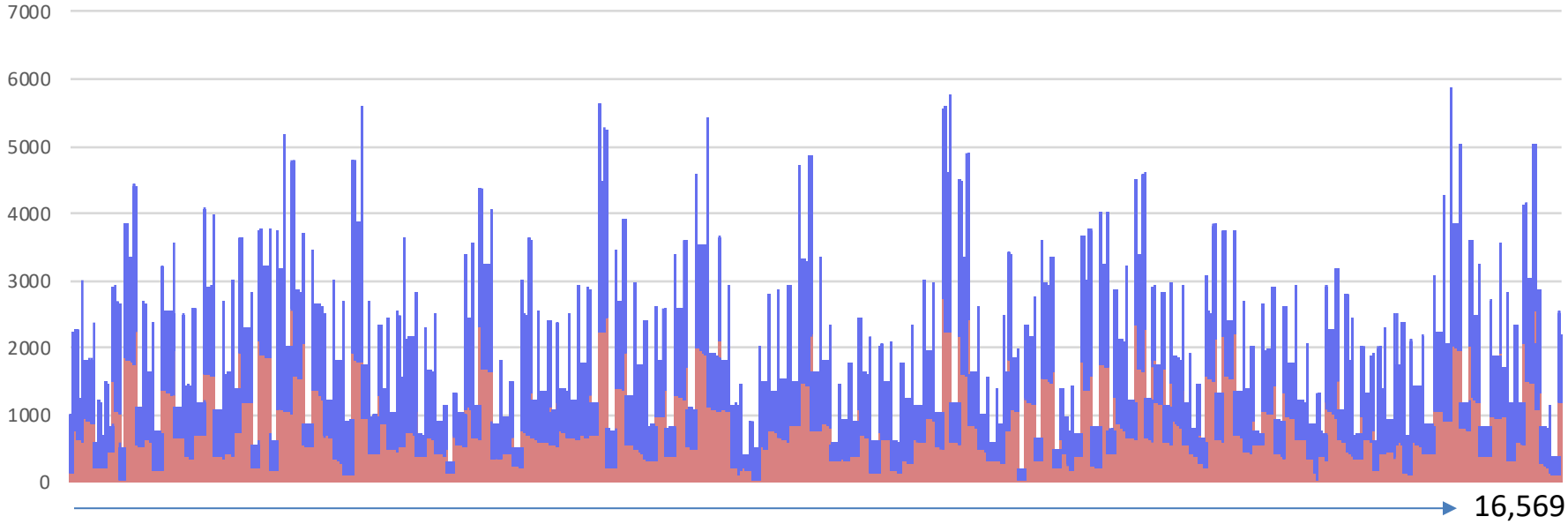
# Applied Biosystems™ Precision ID Whole Genome Panel

## Degraded Samples



Nuclear- No STRs

No HVI/HVII mitochondrial results



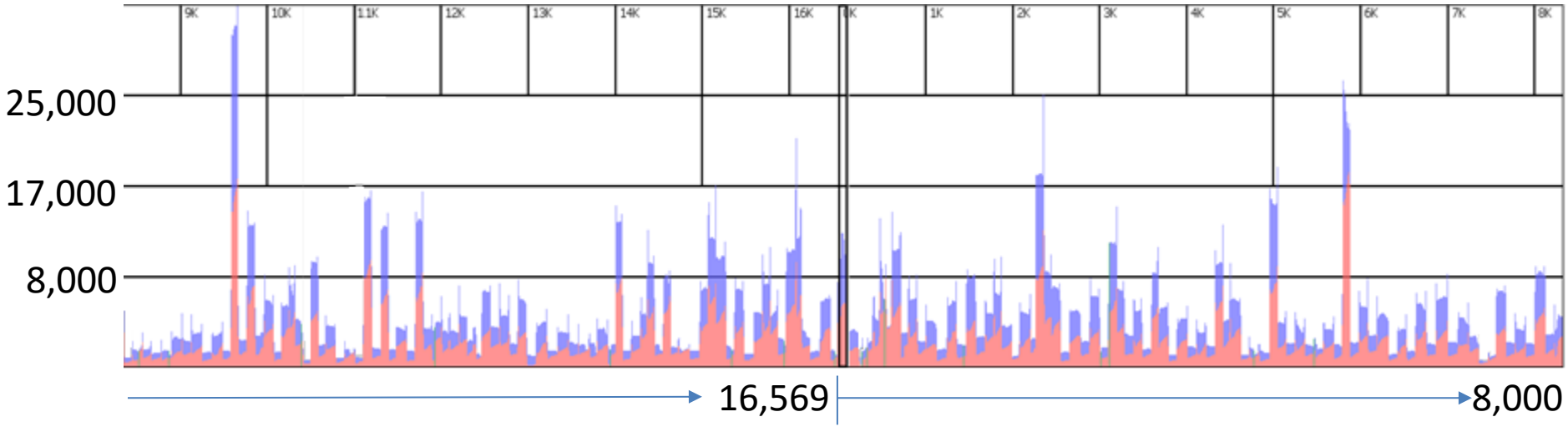

96.7% of Mitochondrial Genome Above 500 Reads

Reference: https://www.videoblocks.com/video/bone-medical-sketch-illustration-hand-drawn-animation-transparent-hhgya6zfirhhqcf3

# Applied Biosystems™ Precision ID Whole Genome Panel

## Degraded Samples

Trio Quant- 0.1 pg/µL nu large  685 pg/µL small  DI: 6850  Nothing Above 180 bp




100% of Mitochondrial Genome Over 500 reads

Reference:https://www.genengnews.com/new-products/ffpe-tissue-analysis/3771

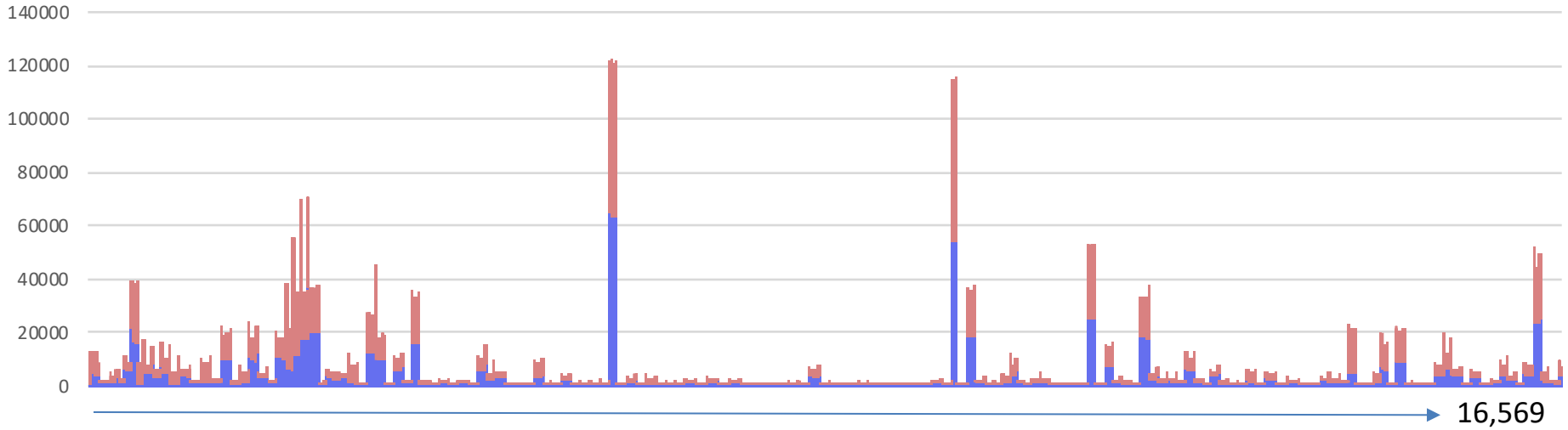
# Applied Biosystems™ Precision ID Whole Genome Panel

## Degraded Samples

Nuclear- No STRs

mtDNA- (200bp) partial HVI

Trio Quant- 0 large target and 231 pg/µL nu small target




91.3% of Mitochondrial Genome Over 500 reads

Reference:https://www.genengnews.com/new-products/ffpe-tissue-analysis/3771

| Sample Name | Coverage % Above 500 | Sanger (CR) Results | MPS (Full Genome) Results |
|---|---|---|---|
| Hair 1 | 100 | ☐ | ☒ |
| Hair 2 | 93 | ☐ | ☐ |
| Hair 3 | 75 | ☐ | ☐ |
| Bone 1 | 99.5 | ☒ | ☒ |
| Bone 2 | 99.4 | ☒ | ☒ |
| Bone 3 | 100 | ☒ | ☒ |
| Bone 4 | 98.8 | ☒ | ☒ |
| Bone 5 | 52* | ☐ | ☒ |
| Bone 6 | 96.7 | ☐ | ☒ |
| Bone 7 | 84 | ☐ | ☒ |
| Bone 8 | N/A | ☐ | ☐ |
| Bone 9 | N/A | ☐ | ☐ |
| Tissue 1 | 91.3 | ☐ | ☒ |
| Tissue 2 | 100 | ☐ | ☒ |
| Tissue 3 | 100 | ☐ | ☒ |
| Nail 1 | 57* | ☒ | ☐ |
| Nail 2 | 100 | ☒ | ☒ |
| Nail 3 | 100 | ☐ | ☒ |
| Nail 4 | 100 | ☒ | ☒ |
| Buccal 1 | 56* | ☒ | ☐ |
| Buccal 2 | 100 | ☒ | ☒ |
| Buccal 3 | 85 | ☒ | ☒ |
| Buccal 4 | 99.2 | ☒ | ☒ |
| | | 9/20= 45% | 17/20= 85% |

# Implementation Plan

- Finalize Analysis Software Validation
- Missing Persons DNA Program Staff will start training later this year
- NDIS package preparation and submission


Reference: https://www.revenuexl.com/blog/end-of-meaningful-use-what-is-next

# Thank you!
# Questions?

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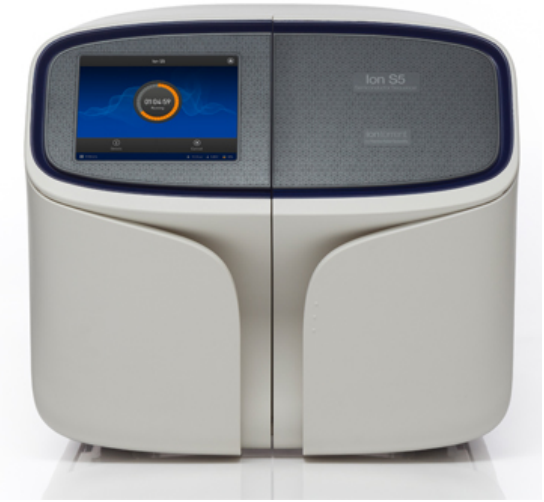
# At a Glance

Library Preparation and Clonal Amplification/ Chip Loading


Sequencing




| | Precision ID Assay |
|---|---|
| **Library Prep Chemistry** | PCR – Adaptor Ligation |
| **Ideal DNA Input** | 100 pg |
| **Samples per Run** | 4-32 |
| **Sequencer** | Ion Torrent S5 |
| **Sequencing Chemistry** | Semiconductor (ion) |
| **Hands On Time** | <2 hours |
| **Full Time (extract to sequence)** | 48 hours (4 work days) |
| **Reads Per Run** | 9-14 Million |
| **Price (per sample*)-** | $ 201.79 (~€ 165) |

*Price per sample will vary depending on the amount of samples that are multiplexed together.

Reference: https://www.thermofisher.com/order/catalog/product/4484177?ICID=search-product
http://www.brand.de/en/products/life-science-products/pcr-disposable-products/pcr-plates/