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# FORENSICS

Validation of a Massively Parallel Sequencing Workflow for Mitochondrial DNA Analysis at UNTHSC Center for Human Identification for Missing Persons and Traditional Casework Analyses

> Jennifer D. Churchill, Maiko Takahashi, Christina Strobl, Dixie Peters, Christina Capt, Walther Parson, Bruce Budowle

### **Application of MPS**



Suhua Zhang<sup>1</sup>, Yong Niu<sup>2</sup>, Yingnan Bian<sup>1</sup>, Rixia Dong<sup>3</sup>, Xiling Liu<sup>1</sup>, Yun Bao<sup>1</sup>, Chao Jin<sup>4</sup> Hancheng Zheng<sup>5</sup> & Chengtao Li (3)<sup>1</sup>

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ORIGINAL ARTICLE

Massively parallel sequencing-enabled mixture analysis of mitochondrial DNA samples

Jennifer D. Churchill<sup>1</sup> 🕞 • Monika Stoliarova<sup>2</sup> • Jonathan L. King<sup>1</sup> • Bruce Budowle<sup>1,3</sup>

Eva Haigh<sup>a</sup>, Joseph Chang<sup>b</sup>, Andrew J. Pakstis<sup>a</sup>

<sup>a</sup> Department of Genetics, Yale University School of Medicine, New Haven, CT, USA <sup>b</sup> Human Identification Group, Thermo Fisher Scientific, South San Francisco, CA, USA



#### Editorial

Transitioning from Forensic Genetics to Forensic Genomics

Manfred Kayser 1,\* and Walther Parson 2,3,\* 😳

Marie-Louise Kampmann, Anders Buchard, Claus Børsting, and Niels Morling

Section of Forensic Genetics. Department of Forensic Medicine. Faculty of Health and Medical Sciences. University of Copenhagen. Copenhagen, Denmark

\*M.K. and A.B. contributed equally to this work.

BioTechniques 61:149-151 (September 2016) doi 10.2144/000114453 Keywords: massively parallel sequencing; FTA cards; DNA long-term storage; next-generation sequencina

### Analysis of Difficult Samples







#### mtDNA is the most successful marker

## Mitochondrial DNA

• Higher copy number per cell compared with the nuclear genome



• Maternal inheritance



#### mtDNA Sequencing with MPS Technologies

#### **Workflow**



Library Preparation







#### Analysis



#### **Primer Panels**



#### mtDNA Sequencing with MPS Technologies

 Entire mtDNA genome can increase discrimination power

> Circos plot illustrating mitochondrial variant data from 283 previously sequenced mitochondrial genomes. Sequence and data analysis of these mitochondrial genomes are described in King et al. 2014.<sup>1</sup>

### mtDNA Sequencing with MPS Technologies

• Increased resolution and quantitative nature of MPS provide avenues for mixture interpretation



Figure taken from Davis et al. 2015

- Limited quantitative information
- Limited haplotype information

- AGGCTGACACTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACACTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACCCTGCGAAAAT AGGCTGACACTGCGAAAAT AGGCTGACACTGCGAAAAT AGGCTGACCCTGCGAAAAT
  - Increased resolution
  - Quantitative results
  - Phasing information
  - Make better use of phylogenetic data

## Adopting a New Technology

• Analysis of the mtGenome with MPS technologies can serve as the first step in transitioning from capillary electrophoresis-based to MPS-based technologies in forensic laboratories









### National Institute of Justice Award 2016-DN-BX-K001

- Evaluation and Implementation of High Throughput Second Generation Sequencing for Mitochondrial DNA Testing in Missing Persons and Forensic Casework at the UNT Center for Human Identification
  - Missing Persons Unit
  - Forensic Unit
    - Hair
  - Initially single source samples







### National Institute of Justice Award 2016-DN-BX-K001

- Purchased equipment
- Installed equipment
- Started training all analysts
  - Reading lists
  - Classroom lectures
  - Demonstration of process
  - Extensive data analyses
  - Benchwork
  - Journal Club



## Validation Studies

- Population studies
- Contamination Assessment
- Concordance
  - Manual and Chef
  - Orthogonal testing (MPS and Sanger)
  - Between laboratories
- Reproducibility/Repeatability
- Sensitivity/Stochastic effects
- Mixtures
- Mock Casework Samples
- Cross Reaction with Other Species







## Mitochondrial Genome Panel

- Multiplex short amplicon system
  - Applied Biosystems Precision ID mtDNA Whole Genome Panel
- Spans entire mitochondrial genome
  - Two multiplex panels
  - Each panel contains 81 primer pairs (plus degenerate primers)
  - Tiled, overlapping pattern
  - Amplicons are  $\leq 175$  bps in length



## Workflow

- Library preparation
  - Manually prepared in a hood
  - Automated on Ion Chef
- Template preparation
  - Completed with Ion Chef
- Massively parallel sequencing
  - Ion S5







## **Bioinformatic Pipeline**

- Raw data analyzed with Ion Torrent Suite Software
  - Downloaded BAM and BAI files
  - Variant calls made with variantCaller plugin; downloaded VCF files
- mitoSAVE
  - Nomenclature
- Manual curation in IGV
  - Phase information
- Verify haplotypes in Haplogrep and Empop
  - Phylogenetic check

#### Evaluate concordance, read depth, strand balance, and noise



## Conclusions

- Continued development of MPS technologies has made implementation into forensic genetic crime labs feasible
- Data from these studies support that this MPS workflow yields reliable results for the analysis of biological evidence
- Our experiences and resources could assist other forensic laboratories considering implementation of MPS
- Procedures developed from our experience will be made available once the process has been completed

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• Thermo Fisher Scientific





**Research** Team



## Questions?

