The Road to Implementation

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Introduction

The Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) allows for amplification of the entire mitochondrial genome with two pools of 81 primer pairs each. This multiplex generates amplicons of 175 base-pairs or less in an attempt to target the DNA of a quality typically encountered in hairs, bones, and teeth. When used with the Ion Chef and Ion S5 (Thermo Fisher Scientific), the library preparation, templating, and sequencing process becomes largely automated, reducing the hands-on time to a total of three pipetting steps. Initial studies with the Precision ID mtDNA Whole Genome Panel evaluated reference samples for concordance and performance with challenged samples, mixed samples, and a dilution series to test the limits of the system. Following successful studies with this multiplex panel, a decision was made to proceed with implementation, which includes an internal validation study, of whole genome mtDNA sequencing in our Missing Persons and Forensic Units.

Materials and Methods

Library Preparation and Massively Parallel Sequencing

The mitochondrial genome was amplified with the Precision ID mtDNA Whole Genome Panel following the manufacturer's recommended protocols [1]. Libraries were prepared both manually with the Precision ID Library Kit and in an automated fashion on the Ion Chef following manufacturer's protocols [1]. Template preparation was completed on the Ion Chef, and sequencing was performed on the Ion S5 following manufacturer's protocols [1].

Data Analysis

Raw sequence data were analyzed using the Torrent Suite software. Data were aligned to an rCRS+80 reference genome to accommodate the multiplex's design [1-2]. Variant calls were generated with the Variant Caller plugin, and the variant call format (VCF) output files generated

by the Variant Caller plugin were used in mitoSAVE [3] to generate haplotype calls in standard forensic nomenclature. A read depth threshold of 10 reads (X) and point heteroplasmy threshold of 0.10 were employed when generating variant calls. Integrative Genomic Viewer (IGV) was used for a visual check of the aligned binary alignment map (BAM) files [4-5]. Finally, a phylogenetic check of the haplotype calls was performed in Haplogrep and Empop [6-7]. Performance metrics, including read depth, strand balance, and noise, were used to evaluate the quality of the sequencing results generated.

Results and Discussion

Developmental validation of the Precision ID mtDNA Whole Genome Panel, Ion Chef, and Ion S5 system included studies of population samples, concordance, reproducibility, sensitivity, stochastic effects, potential for contamination, mock casework samples, mixtures, and cross reaction with other species. Data from these developmental validation studies were leveraged for training and internal validation studies.

Training for forensic analysts included 6 one-hour classroom style lectures along with up-close real-time videos of the entire workflow. These videos allowed analysts to experience the procedure and reinforce what they had just learned. Then hands-on training of data analysis and the entire workflow at the lab bench ensued. As massively parallel sequencing is not yet widely used in forensic genetic casework, guidance on training materials and processes, development of SOPs, workflow considerations, the steps for internal validation studies, and the experience of implementation will be provided to facilitate other laboratories who decide to implement this technology.

Conclusions

The continued development of massively parallel sequencing technologies supported by numerous studies throughout the genetic and forensic communities has made implementation of this technology into forensic genetic crime labs feasible. Our experiences and resources developed along the road to implementation could assist other forensic laboratories considering implementation of massively parallel sequencing.

Acknowledgements

This work was supported in part by award no. 2016-DN-BX-K001, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

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