Massively Parallel Sequencing of the Mitochondrial Genome in Casework-type Samples

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Introduction

The benefits afforded by massively parallel sequencing (MPS) technologies have been studied in detail over the last several years. For mitochondrial DNA (mtDNA) sequencing, MPS makes it feasible for forensic laboratories to analyze the entire mitochondrial genome (mtGenome), which provides an opportunity for an increase in discrimination power and phylogenetic resolution. Additionally, the quantitative aspects of MPS technologies provide new opportunities to deconvolve mixed samples in mtDNA analysis. Recent developments, such as updates to SWGDAM Guidelines and NDIS approval of MPS data from two MPS workflows, demonstrate the growing acceptance of MPS technologies in the forensic community. Early adopters of MPS technologies have started validating and implementing MPS workflows in their labs. The Missing Persons and Forensic Units at UNTHSC's Center for Human Identification (UNTCHI) became one of these early adopters with the decision to implement mtGenome testing of biological evidence using a MPS workflow.

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 and aligned to an rCRS+80 reference genome to accommodate the multiplex's design [1-2]. The HIDGenotyper v2.1 plugin and Converge Software v2.1 were used to generate variant calls in standard forensic nomenclature. Converge default thresholds of 20 reads (X) and 10% for point heteroplasmies, 20% for insertions, and 30% for deletions were used when generating variant calls. Performance metrics, including concordance, read depth, strand balance, and noise, were used to evaluate the quality of the sequencing results generated.

Results and Discussion

As part of this implementation process, UNTCHI performed validation studies on a MPS workflow that consists of the Precision ID mtDNA Whole Genome Panel, the Ion Chef, and the Ion S5 (Thermo Fisher Scientific). Known and non-probative evidence and evidence-type samples that include blood, saliva, semen, buccal swabs, hairs, and bones were sequenced. These samples included a range of high-quality, high-quantity samples (i.e., manufacturer recommended amount of input DNA) to low-quality, low-quantity samples (i.e., unquantifiable amount of nuclear DNA). Mixtures with contributor ratios ranging from 1:2 to

1:20 were also sequenced. Performance metrics, including read depth, strand balance, and noise, were evaluated for each of these samples. A subset of these samples was processed with both automated and manually prepared libraries for a comparison of the performance between the two library preparation methods. Haplotype calls were compared to previously generated Sanger sequencing results to evaluate concordance and the increase in information afforded by MPS. Mixtures were separated into component haplotypes and compared to the single-source known contributor haplotypes.

Conclusions

Data generated from the evaluation of these samples support that this MPS workflow yields reliable results for the analysis of biological evidence, and the experience and resources gained from UNTCHI's implementation could assist other forensic laboratories in their own implementation process.

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