Evaluation of a Short-amplicon Multiplex for the Mitochondrial Genome on the Ion PGM

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Introduction

The mitochondrial genomes of 31 individuals (African, Hispanic, and Caucasian descent) were sequenced with the original and Hq™ sequencing chemistries. • Long PCR primers (Gunnarsdóttir et al. 2011) were used for amplification of the mitochondrial genome.

Materials and Methods

Original and Ion Torren Hi-Q™ Sequencing Chemistry

The mitochondrial genomes of 31 individuals (African, Hispanic, and Caucasian descent) were sequenced with the original and Hq™ sequencing chemistries. • Long PCR primers (Gunnarsdóttir et al. 2011) were used for amplification of the mitochondrial genome.

Evaluation of Ion Torren’s New Mitochondrial Genome Panel

Thirty-one individuals were sequenced with the mitochondrial genome panel, and sequence concordance, coverage, and strand balance were used to evaluate the quality and reliability of the data produced. Sequence data generated on these individuals also were evaluated for possible amplicon drop out. Analysis showed that haplotype calls for these samples were concordant with whole mitochondrial genome data generated by Ion PGM (Figure 4). Concordance ranged from 97% to 98.8%. Reads were generated from both strands of the DNA. Amplicon drop out was minimal and no more than what would be expected given the whole genome data being amplified (Table 1). These results indicate robust and accurate data were generated with minimal amplicon drop out. A serial dilution was performed for three individuals with input DNA ranging from 1 ng to 1 pg. Results demonstrate the sensitivity of this mitochondrial multiplex (Figure 7).

Results

Figure 2. Both enzymes produced similar relative locus performance (RUP) across the entire mitochondrial genome. RUP was used to account for any variability introduced by running samples on different chips. Higher internal RUP was observed with the Hq™-sequencing chemistry.

Figure 3. Both enzymes produced similar strand balance across the entire mitochondrial genome with the Hq™ enzyme tending to provide more coverage of the reverse strand.

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Figure 3. Both enzymes produced similar strand balance across the entire mitochondrial genome with the Hq™ enzyme tending to provide more coverage of the reverse strand.

Figure 4. BAM files of sequence data across the entire mitochondrial genome with the original enzyme (top) and Hq™ enzyme (bottom) aligned in IGV.

Figure 5. The delta of the deletion ratios for each position across the mitochondrial genome (A). Delta was calculated by subtracting the deletion ratio of the Hq™ enzyme from the deletion ratio of the original enzyme. Positive delta values represent positions where the denominator (expected allele) was larger than the numerator (observed allele).

Figure 6. BAM files of sequence data across the entire mitochondrial genome generated with the short-amplicon mitochondrial genome panel (top) and long PCR (bottom) aligned in IGV.

Figure 7. Results from the portion of the serial dilution analyses on three samples with input DNA ranging from one ng to 1pg. Results showed robust mating call ability (A) and concordant across the entire genome (B). The overall success rate was exceedingly high. The results for the three samples are summarized as follows:

Sample 1: Haplotypes were concordant across the entire dilution series.

Sample 2: No missing variants.

Sample 3: Three variants dropped out in the 0.1 pg sample.

All variants were present, but at a read depth <10x.

The amplicon that covers this region tends to be a low performing amplicon in the 31-sampled set.

Conclusions

A comparison of the data generated with the original and Hq™ sequencing chemistry was performed. Results for haplotype calls, coverage, strand balance and noise levels demonstrated that the Hq™ enzyme performed similarly and generated accurate and reliable sequence results. In addition, the Hq™-enzyme displayed an improved ability to sequence through homopolymeric regions.

Using the multiplex mitochondrial genome panel and the Hq™ sequencing chemistry, data in this study support that reliable mitochondrial genome sequencing can be performed with the Ion PGM™ system. Additionally, when used with the Ion Chemos, this multiplex mitochondrial genome panel generates a workflow with enough efficiency for forensic laboratories to consider analysis of the entire mitochondrial genome. The evaluation of this new mitochondrial genome multiple panel also demonstrates that it has a high sensitivity of detection.

These promising results support continued development of the MPS technology for forensic analyses. Future work will include the addition of degenetators primers to the mitochondrial genome panel primer set, population studies, and continued validation studies of the mitochondrial genome panel.

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References