FORENSIC MITOCHONDRIAL DNA TYPING USING THE ION TORRENT PGM™

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Mitochondrial DNA (mtDNA) typing provides genetic information on maternally related individuals, such as in identification of human remains cases, as well as for direct associations of an individual and a crime scene sample. Sanger sequencing has been a standard method for most typing in forensic applications. However, with the advent of second generation sequencing (SGS) and its more comprehensive coverage, forensic applications should be considered. The development of high throughput "personal sequencers" allow for sequencing to be strategically focused on a smaller genome or selected panels of markers in a more efficient and cost amenable fashion. These personal sequencers generate relative large amounts data compared with traditional sequencing. The sequencers also have advantages of short run time and lower instrumentation cost. One of the personal sequencers available on the market is the Ion Torrent Personal Genome Machine (PGM™) (Life Technologies). The PGM is a platform of non-optical sequencing on CMOS integrated circuits that detects small changes in pH, due to release of H+ during addition of a nucleotide to the growing strand. For PGM, the output currently is 1GB using 318 Chip with a 2 hour run time.

Developmental and validation studies are underway to use the PGM platform for forensic mtDNA analyses. The goal is development of efficient mtDNA testing protocols that can encompass the entire mitochondrial genome and, thus, provide higher resolution and discrimination power than is currently possible with only sequencing portions of the non-coding region of the mitochondrial genome.

The primary facets of the studies are:
1) Effectiveness of sequencing of targets generated by specific amplicon or Whole Genome Amplification (WGA)
2) Assessment of throughput, coverage, and bar coding capability
3) Concordance of results with Sanger sequencing results
4) Determination of reliability and reproducibility
5) Assess and impact of increased resolution of heteroplasmy
6) Development of interpretation guidelines for single source and mixture samples, heteroplasmy, homopolymeric stretch regions, and potential chimeras
7) Determination and development of procedure to increase sensitivity of detection
8) Evaluation of performance with challenged samples, such as bones, teeth and hair

The general capability of sequencing mtDNA with the PGM is demonstrated. The progress on these studies will be presented to provide insight to the forensic science community on the near term applications and long term potential utility of SGS for forensic casework analyses. The PGM generates sequence data rapidly with relatively high coverage compared with other SGS platforms and significantly greater coverage than current Sanger sequencing methods. Effective mtDNA typing protocols will provide more detailed information, allowing for more accurate associations. ⌘