A53 Low-Level Variant Detection in Mitochondrial DNA Using the Illumina® GA IIx Next-Generation Sequencing (NGS) Platform

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After attending this presentation, attendees will be more familiar with the concept of heteroplasmy and interpretation bias drawn from mitochondrial DNA sequence data using current sequencing methodologies. An overview of the Illumina® Next-Generation Sequencing (NGS) chemistry and workflow will be presented. Data elucidating depth of coverage required for variant detection at or below a 1% threshold will be shown. Per sample cost, training issues and implementation strategies will also be mentioned.

This presentation will affect the forensic science community by suggesting an alternative method for mtDNA sequence analysis, one that is able to reliably detect low-level variants in mtDNA down to the single molecule level.

Heteroplasmy is defined as the presence of more than one mitochondrial DNA (mtDNA) type within an individual (Melton 2004).[1] These differing mtDNA types may occur between populations within an individual, within a single cell, or within a single mitochondrion. Furthermore, heteroplasmy has been observed in two distinct forms including sequence and length heteroplasmy. Sequence heteroplasmy occurs when two nucleotides are present at a single nucleotide position, while length heteroplasmy occurs when two different lengths of a homopolymeric C-stretch are present within the mtDNA sequence. Higher mutation rates (Bogenhagen 1999)[2] and fewer DNA repair mechanisms than nuclear DNA (Kunkel 1981)[3] lend credence to the idea that millions of mtDNA molecules within an individual are not likely to have a single, uniform mtDNA type. As a result, it is presumed that all individuals are heteroplasmic at some level and therefore, mtDNA sequences from single source samples are a mixture of mtDNA types. Heteroplasmy may go unnoticed given the limits of detection of traditional Sanger sequencing methods, whereby a single sequence is generated from amplified mtDNA products. Failing to detect heteroplasmy gives bias to interpretation of mtDNA sequence data. With the advent of sequencing-by-synthesis technologies, it is possible to resolve and quantify mixtures of mtDNA at much lower levels than current technologies (Andréasson *et al.* 2006).[4] These findings suggest that sequencing-by-synthesis methods may be an attractive alternative to current methods used in Forensic laboratories by allowing for the detection heteroplasmy below a threshold of 1%. The Illumina® GA_{IIx} is a massively-parallel sequencing platform which utilizes a unique chemistry that is unlike most pyrosequencing technologies currently on the market. The Illumina® GA_{IIx} utilizes a proprietary flow cell that is covered with a dense lawn of primers which will bind to flanking adapters incorporated into amplified DNA products (Illumina® 2010).[5] Clonal amplification of captured products results in tens of millions of clusters per square centimeter of the flow cell. Each cluster is then sequenced and analyzed independently of one another. This allows for the generation of sequence data on a single molecule scale.

In this effort, the Illumina® GA_{IIx} was evaluated for mixture analysis of mtDNA. Initially, DNA was extracted from forensically relevant sample types including donated hair, buccal, and blood samples. The DNA was amplified using a novel amplification strategy described by Bintz *et al.*, and reference sequences were obtained from the mtDNA HV region of all 20 donors using Sanger sequencing methods. Artificial mixtures were then prepared using distinct templates from the samples described above in ratios of 99%/1%, 98%/2%, and 95%/5%. Multiplex assays were designed using the resulting mixtures. Resulting data shows the reliable detection of variants at a target level of 1%, which is significantly lower than the 10% threshold of current methods. Additionally, we were able to quantify the depth of coverage needed to detect variants at a level of 1% at an average of 250X coverage from approximately 50 individuals per Roche GS-Junior run.

References:

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4. Andréasson, H, Nilsson, M, Budowle, B, Frisk, S, and Allen, M. Quantification of mtDNA mixtures in forensic evidence material using pyrosequencing. *International Journal of Legal Medicine*. 120:383-390.
5. Illumina. *Illumina Sequencing: Run Quality and Troubleshooting*. 2010

Next Generation Sequencing, Mitochondrial DNA, Heteroplasmy

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