NEXT GENERATION MITOCHONDRIAL DNA SEQUENCING AT THE FBI LABORATORY

Michael D. Brandhagen, Lilliana Moreno, Odile Loreille, Anthony Onorato, Thomas Callaghan, Jodi Irwin, Federal Bureau of Investigation, Laboratory Division

Next Generation Sequencing (NGS) offers the capability to generate larger, more informative genetic data sets from single samples at higher throughput and at an overall lower cost per nucleotide than traditional capillary electrophoresis-based methods. Given this potential, the Federal Bureau of Investigation is pursuing NGS for DNA casework application, with near term mitochondrial DNA casework application a primary focus. As mitochondrial DNA testing is already DNA sequencing-based, it presents a logical first step in the Laboratory's implementation of this new technology. Furthermore, NGS makes it technically feasible to recover entire mitochondrial genome data from the most limited evidentiary material, greatly improving the power of mtDNA testing.

However, NGS introduces some unique issues that must be overcome before the full potential of mtDNA sequencing can be realized in casework. These include genetic privacy issues associated with sequencing of the entire mtGenome, the need for appropriate reference population data for evidence interpretation, and the need for specific Scientific Working Group on DNA Methods (SWGDAM) guidelines that address NGS. The FBI laboratory is approaching NGS with these issues in mind, and thus current efforts are focused on the evaluation and validation of mitochondrial DNA control region (mtDNA CR) assays. Though these assays do not take full advantage of the potential of NGS, they stand to both extend the lower limits of sample quality from which probative mitochondrial DNA data can be recovered and, importantly, facilitate the acceptance of NGS technology in the courtroom.

This presentation will address the FBI Laboratory's approach to NGS, and detail data from the optimization and validation of a mtDNA CR NGS workflow using the Promega PowerSeq™ mtDNA CR assay, the Ilumina TruSeq Library prep, the Illumina MiSeq™, and CLC Genomics Workbench data analysis software, for use in casework. Data from reproducibility, sensitivity, concordance, and mixture studies will be presented, along with guidelines for assessing data quality from both high quality (blood and buccal swabs) and low quality samples (e.g. hair and calcified tissue). In addition, data from preliminary NGS mtGenome assay optimization and validation experiments will be discussed.