A164 Next Generation Sequencing Applications for SNPs and Mitochondrial DNA in Human Identification

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After attending this presentation, attendees will gain an understanding of comprehensive laboratory and bioinformatic workflows for obtaining mitochondrial and SNP genotypes using next-generation sequencing technology.

This presentation will impact the forensic science community by giving an understanding of the potential use of next generation sequencing for human identification applications.

Next-generation sequencing technology is a subject area in which the forensic community has previously done little research. However, this presentation shows that using genomic DNA or mitochondrial DNA extracted from bone, blood, buccal swab, or other forensic samples, one can obtain mitochondrial and/or SNP genotypes for up to 96 individuals in a single next-generation sequencing run.

The field of human identification has been dominated by capillary electrophoresis-based (CE) STR fragment analysis. There has also been a minor effort to sequence the hypervariable regions I/II of the mitochondrial genome by CE. The low throughput of CE sequencing makes it difficult to incorporate complex DNA testing into routine procedure for criminal labs. Next-generation DNA sequencing technologies have advanced dramatically in recent years, and the costs have been reduced enough to make adoption of next-generation sequencing more attractive to the forensic community and criminal labs. Laboratories that adopt a next-generation sequencing approach are capable of generating data that can be used to produce a more detailed genomic and phenotypic profile than the current STR approach at less cost than traditional sequencing or SNP assays.

The whole 16kb mitochondrial genome can be sequenced in one next-generation sequencing run. If sequenced on CE, 64 separate reactions would be necessary (assuming 500bp amplicons and forward/reverse sequences). In fact, the new higher throughput capacities enable whole mitochondrial genome sequencing of up to 96 individuals in a single sequencing run.

To test the feasibility of this idea, an assay system containing 32 fusion adaptors with a short sequence tag made of different combinations of nucleotides attached to an adaptor (barcode) was developed. The whole mitochondrial genome was amplified with 2 PCRs each yielding overlapping 8-9kb amplicons. The two PCR products were then combined, sheared, and ligated to an adapter and a fusion barcoded adaptor. The PCR products from each individual can then be pooled and sequenced in a single sequencing run. Additionally, for sequencing more compromised samples, a 2 PCR mitochondrial mini amplicon system consisting of two multiplexes of five primer sets spanning the mitochondrial control region was tested.

To demonstrate feasibilty for an SNP assay, a panel of 106 autosomal and 33 Y chromosome SNPs selected from publically available datasets was constructed. A single PCR multiplex for ~200bp amplicons covering the 139 SNP loci was generated. The PCR products were ligated to adaptors, and barcoded libraries from 32 individuals were pooled and sequenced in one sequencing run and compared to reference genotypes.

The high capacity of the next-generation sequencing technologies makes combining many of the currently used human identification methods possible. Multiplexes of any combination of STRs, phenotypic SNPs, autosomal identification SNPs, lineage SNPs, Y SNPs, Indels, mitochondrial sequences, or SNPs are possible in one reaction. Inclusion of historical markers would allow for a gradual shift to other markers while at the same time keeping continuity with current forensic data sets.

Sequencing, Mitochondrial, SNP

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