A95 A Sensitive Multiplex PCR Based Next- Generation Sequencing Assay for Resolution of Mixtures and Analysis of Forensic Samples

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After attending this presentation, attendees will have a broader understanding of the 454 next-generation sequencing technology and the applications for forensic analysis, specifically for mtDNA mixture analysis.

This presentation will impact the forensic science community by providing an overview of a new, sensitive multiplex PCR assay for analysis of forensically relevant samples including mtDNA mixtures and heteroplasmy using the 454 Next-generation sequencing technology.

Next-generation sequencing (NGS) technologies have proven to be a powerful tool for research and clinical applications and have the potential to revolutionize forensic DNA analysis. NGS technologies offer a high-throughput solution for parallel sequencing of thousands to millions of sequences and can be used for de novo sequencing of small whole genomes or direct sequencing of DNA products generated by PCR. While several NGS technologies are available, the 454 sequencing technology appears to be the most suitable for forensic applications because it can directly sequence 400-500 bp amplicons. The 454 Genome Sequencer is a scalable, highly parallel pyrosequencing system that uses emulsion- based PCR for "clonal" amplification of single DNA sequences. The "clonal sequencing" aspect of this technology allows unambiguous allele resolution and provides for quantitative detection of variants present in less than 1% in a mixture. The 454 sequencing technology has been successfully used to analyze mixtures in clinical samples. Recently, the feasibility of 454 sequencing technology for analysis of mixed DNA samples similar to those encountered in forensic evidence has been demonstrated by sequencing mtDNA and nuclear STR markers. However, each marker was amplified in single-plex and significantly more DNA was required than is typically available in forensic cases. Thus, before this technology can be routinely used for analysis of forensic samples which are often limited and/or degraded, assays which require much less DNA need to be developed for use with NGS technology.

To greatly reduce the amount of DNA consumed for NGS, a multiplex PCR assay for parallel sequencing of mitochondrial and nuclear DNA markers which can be used for analysis of pooled forensic DNA samples has been developed. The use of a multiplex PCR assay for targeted resequencing of multiple mtDNA and STR markers is essential for forensic applications whereby DNA is often limited. Fusion primers containing unique multiplex identifiers (MID) are incorporated in the PCR primers containing target and 454-sequences and used to amplify individual samples for pooling and sequencing in a single run, thereby increasing sample throughput and reducing per run cost. The results from a sensitivity and mixture study demonstrating the utility of this NGS technology for analysis of forensic samples and the 454 sequencing data from heteroplasmic samples will be presented. Preliminary results show that the starting amount of DNA can be significantly reduced by using the multiplex PCR assay, similar to conventional forensic methods.

This multiplex PCR based NGS assay will allow the practitioner a start to finish method for sequencing mtDNA and nuclear STRs in a single run. This system can be used for the resolution of mixed or heteroplasmic samples commonly encountered in forensic cases and would therefore provide forensic laboratories with a more sensitive alternative to standard forensic assays.

Next Generation Sequencing, mtDNA, Mixtures and Heteroplasmy

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