A159 Whole Mitochondrial Genome Probe Capture Next Generation Sequencing Method for Analysis of Limited and Degraded DNA Samples

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After attending this presentation, attendees will be informed about a new method for enriching and sequencing the whole mitochondrial DNA genome for analysis of limited, mixed, and degraded samples using next generation sequencing technology.

This presentation will impact the forensic science community by demonstrating a high throughput, next generation sequencing method for the mitochondrial genome that increases the discrimination power over current methods. Further, the new method introduces a mechanical shearing technique for DNA fragmentation and a liquid-based probe capture step that facilitates the processing of highly degraded samples.

Degraded and/or size-limited DNA samples are often encountered in forensic casework. The application of mini Short Tandem Repeat (STR) and bi-allelic Single-Nucleotide Polymorphisms (SNPs) have increased the success of analyzing these samples; however, these methods target nuclear DNA and, although there is a clear advantage in doing so (high discrimination power), degraded samples often cannot be effectively analyzed. The use of mitochondrial DNA (mtDNA) is beneficial in these cases due to its high copy number and small size. Yet, its highly conserved genome lacks discrimination potential.

The application of next generation sequencing technology allows for the analysis of the entire mitochondrial genome, which compensates for limitations of current mtDNA analysis methods. A probe capture 454 next generation sequencing assay was developed for target enrichment and deep sequencing of the whole mitochondrial genome. The liquid phase probe capture step in the developed method was included into the assay design to enrich for mtDNA and to eliminate the need for amplification primers that target the sample DNA. By doing so, the designed assay has the potential to analyze highly degraded samples while bypassing amplification complications. To further improve the assay's capability of processing degraded samples, a mechanical shearing fragmentation method, independent of sample quality, was incorporated into the assay. The goal of this project was to optimize and validate this assay for use on degraded, mixed, and/or size-limited samples.

The library preparation, the first step in the whole mitochondrial DNA sequencing assay, begins with the fragmentation of the sample DNA into short fragments, typically ~400-500bp. Controlled enzymatic digestion is routinely used for this step in genomic studies, but this approach presents difficulties in analyzing limited and degraded samples. For forensic samples, it is important the assay be capable of processing degraded and limited samples in the same way it processes pristine samples. This would allow a crime laboratory to use a single protocol for any sample it analyzes. To achieve this goal, a fragmentation method using mechanical shearing was optimized and implemented. The Covaris® mechanical shearing uses ultrasonic waves that form cavitation bubbles that cut DNA as they implode. This unique Adaptive Focused Acoustics™ technology has an energy focal point inside the sample tube, making the technology accurate, reproducible, and quantity independent. To test if this mechanical shearing technology was also quality independent, DNA samples naturally or artificially degraded to different levels were treated with Covaris®. The results showed fragments of uniform size regardless of the initial state of degradation of the sample DNA. In addition to fragmentation, the initial PCR of library preparation was modified for optimal amplification of limited samples. This optimized method was then tested for its ability to process forensically relevant samples.

Results will be presented showing successful analysis of limited, mixed, and degraded samples processed using the optimized fragmentation, capture, and next generation sequencing method. Results from a sensitivity study will be presented demonstrating successful fragmentation and capture of limited DNA amounts with 100% mtDNA sequence coverage. The high success observed for even the lowest DNA amount tested (100pg), which is significantly lower than the manufacturer-recommended DNA sample amount of 1µg, suggests that even lower starting DNA amounts may be successfully analyzed. A mixture study resulted in successfully distinguishing the two sequenced profiles in a 5% mixture (the lowest mixture ratio tested). Finally, degraded DNA sample libraries were successfully created showing proof of concept for the optimized mechanical shearing fragmentation method for processing samples independent of

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quantity and quality. The results suggest the optimized method has high mixture and concentration sensitivity and has the potential to process degraded samples. The optimized whole mitochondrial DNA sequencing assay shows promising results for further validation and testing on degraded DNA samples encountered in forensic casework.

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