A160 Whole Mitochondrial Genome Sequencing Using Probe Capture and 454 Sequencing Technology

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After attending this presentation, attendees will gain deeper knowledge of sequence capture for enriching mitochondrial DNA and next generation sequencing using 454.

This presentation will impact the forensic science community by introducing a novel capture method for sequencing the entire mitochondrial genome of degraded or limiting samples.

Mitochondrial DNA (mtDNA) analysis is most useful in forensic cases when samples are degraded and nuclear STR testing cannot produce a complete discriminating profile. The most widely used approach for mtDNA analysis is sequencing the hypervariable regions (HVI/HVII) by Sanger sequencing. The maternal inheritance pattern of mtDNA and sequencing information from only the HV regions provides limited discrimination power, particularly in the Caucasian population due to a few common types. Sanger sequencing is also limiting in that it often fails to detect and resolve low level mixtures, as well as low level heteroplasmy; both common observations in forensic mitochondrial samples. The development of a method for whole mitochondrial genome sequencing using a liquid phase hybridization probe capture strategy followed by sequencing using 454 Next Generation Sequencing (NGS) to overcome these limitations will be described. The hybridization probe capture technique allows the entire mitochondrial sequence to be captured for analysis regardless of fragment size by exploiting a very large number of capture probes designed directly from the mitochondrial genome. Utilizing 454 NGS after capture yields tens to hundreds of thousands of sequence reads making it possible to detect mixtures, including heteroplasmy at lower levels, than current Sanger sequencing methods. Moreover, highly degraded DNA samples already consist of small DNA fragments and can be directly subjected to the capture and sequencing analysis.

The Nimblegen SeqCap EZ platform was chosen as the capture platform due to its extensive tiling design and ability to efficiently incorporate hundreds of thousands of capture probes. To increase the specificity of the probes, the circular nature of mtDNA, and the high density and distribution of polymorphisms was considered in the design strategy. The probes were also designed to exclude the capture of known nuclear pseudogenes. The final design directly targets 99.99% of the mitochondrial genome with unique probes.

Results show that 100% of the mitochondrial genome of all samples was captured with coverage adequate to yield unambiguous sequence assignments with an average on target capture rate of 75%. All SNPs previously detected by Sanger sequencing were also detected by 454 sequencing in all samples. Multiple samples have been tested to evaluate the specificity of the assay. The sensitivity of the method was tested by reducing the starting amount of DNA to forensically relevant DNA levels (<1ng sample DNA) with no loss in sequencing accuracy. The method also achieved resolution of mixtures below the limits of Sanger sequencing (<10%). To improve efficiency, the probe capture hybridization time was reduced from the manufacturer's recommendation of three days to one day. This greatly improves the throughput of the capture method, without affecting the on target capture rate, or accuracy of the capture probes.

Current studies focus on improving the sensitivity and robustness of the method. To analyze samples from more diverse populations and then investigate forensically relevant samples is propsed in this study. These samples include ones with even more limited total DNA, and samples which have degraded beyond the limit of conventional STR analysis. In conclusion, a method for whole mitochondrial genome capture followed by NGS which can be applied to the field of forensic science has been successfully developed.

Mitochondrial DNA, 454, Sequence Capture

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