MITOCHONDRIAL GENOME AND NUCLEAR SNP PROBE CAPTURE NEXT-GENERATION SEQUENCING SYSTEM FOR ANALYZING DEGRADED AND MIXED DNA SAMPLES

Cassandra Calloway[1,2], Nikhil Bose[1,2], Rachel Gordon[1], Cassandra Taylor[1,2], Shelly Shih[1,2], Guillermina Almada[1,2], George, Sensabaugh[3], and Henry Erlich[1]

[1] Children's Hospital Oakland Research Institute, University of California San Francisco Benioff Children's Hospital

[2] Forensic Graduate Group, University of California

[3]University of California, Berkeley

Forensic biological samples that are highly compromised (degraded, limited, or mixed) can often fail conventional STR genotyping. In cases where nuclear DNA is degraded, SNPs can serve as a suitable alternative marker for nuclear markers since the targeted variation is a single base pair. However, conventional PCR based SNP analysis methods are not suitable when samples are highly degraded and PCR primer binding sites are not intact. An alternative strategy is analyzing mitochondrial DNA (mtDNA) especially when nuclear DNA is highly degraded and limited (such as in telogen hairs) since the mitochondrial genome occurs in high copy number per cell. However, conventional mtDNA sequencing methods such as Sanger Sequencing are limiting in that it often fails to detect low level heteroplasmy as well as mixtures which are common in forensic samples. Next-generation sequencing (NGS) methods on the other hand have the potential to overcome many of the limitations of conventional methods used for analyzing mtDNA and SNP markers due to its high throughput massively parallel clonal sequencing nature. In conjunction with using NGS, we have developed probe capture enrichment systems targeting the entire mitochondrial genome and over 450 nuclear polymorphisms in highly degraded and mixed samples. This approach uses DNA probes to enrich targeted regions from randomly fragmented DNA libraries for clonal, massively parallel sequencing, thereby maximizing recovery of short DNA fragments characteristic of forensic samples. Using these capture assays, we have demonstrated that highly degraded DNA ranging from 50 to 150 bases can be analyzed with over 90% target coverage (read depth >100X). Telogen hair containing mtDNA and limited nuclear DNA was analyzed yielding full mtgenome profiles, and partial, but highly informative SNP profiles. Furthermore, we have utilized the clonal nature of NGS to analyze nuclear and mitochondrial DNA mixtures as low as 10%, and mitochondrial germline heteroplasmy as low as 5%.