DEVELOPMENTAL VALIDATION OF A PROBE CAPTURE NGS SYSTEM FOR ANALYSIS OF WHOLE MITOCHONDRIAL GENOME OF FORENSICALLY CHALLENGING SAMPLES

Shelly Shih[1], Rachel Gordon[2], Daniela Cuenca[2], George Sensabaugh[3], Henry Erlich[3], and Cassandra Calloway[3]
[1]Children's Hospital Oakland Research Institute
[2]Jan Bashinski DNA Laboratory, California Department of Justice
[3]University of California, Berkeley, School of Public Health

Mitochondrial (mt/mito) DNA analysis has become a particularly useful tool for testing samples containing insufficient nuclear DNA for conventional STR analysis. In highly compromised samples, DNA degradation can reduce the presence of two intact primer binding sites on the template DNA fragments, often resulting in failure of PCR amplification of the targeted genetic markers. To allow the analysis of these challenging case-type samples, we have developed and validated a probe capture next-generation sequencing (NGS) system for target enrichment of mtDNA from highly degraded samples. Our method utilizes biotinylated DNA probes in a tiling pattern across the mito-genome to hybridize to all mitochondrial haplotypes, NGS to achieve ample sequence read depth, and digital read counts to provide sensitive and quantitative detection of minor sequences at mixed base positions. We have completed a developmental validation of the probe capture NGS system following the standard tests in the Scientific Working Group on DNA Analysis Methods (SWGDAM) validation guidelines. We demonstrated that the probe capture NGS system is sensitive, robust, precise, and accurate for sequencing of the whole mito-genome from reference and simulated case samples. Our data showed that this system is sensitive in detecting minor contributor sequences in a 95:5 two-person mixture at input DNA of 1 ng. This system is capable of generating valuable sequence data for case-type samples including shed hairs, cut hairs, solid tissues, and touch DNA recovered from spent brass cartridges.