MASSIVELY PARALLEL SEQUENCING OF MULTIPLEX SHORT AMPLICONS OF mtDNA FROM CHALLENGED FORENSIC SAMPLES

Monika Stoljarova[1,2], Jonathan L. King[1], Jennifer D. Churchill[1], Bruce Budowle[1,3]

[1]Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center
[2]Centre for Biology of Integrated Systems, Tallinn University of Technology
[3]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University

One of the major challenges in forensic DNA investigations is analyzing damaged/degraded and low copy number samples that often have limited success with STR typing. With samples that fail to yield results by STR typing, mitochondrial DNA (mtDNA) becomes a viable alternative as there are a thousand times more copies of mtDNA per cell compared with nuclear genome. Forensic studies have focused on mtDNA sequencing of hypervariable regions I and II (HVI and HVII, respectively) because these regions contain a concentration of variations. Sequencing beyond HVI and HVII is rarely attempted because the current methodology is labor intensive, time-consuming, and costly. However, it has been reported that approximately 75% of mtDNA variation resides within the coding region. Thus, the full power of discrimination of mtDNA is not realized.

The aim of this study is to develop a proof-of-concept multiplex PCR assay comprised of short amplicons (≤ 200 bp-long) at targeted sites on the mtDNA genome using massively parallel sequencing (MPS). Nine regions within the coding region were chosen for multiplex primer assay design. *In silico* analysis showed that the addition of these nine regions can increase discrimination power beyond sequencing of HVI and HVII to 17.4% in Caucasians. Previously established primers for HVI and HVII by UNT Center for Human Identification (UNTCHI) were added to the designed multiplex primer set for amplification of 170-210 bp long mtDNA fragments. Whole-blood Caucasian samples were amplified according to an optimized mtDNA Amplification protocol from UNTCHI. Amplified products were successfully sequenced using the MPS platform Personal Genome Machine (PGM, Ion Torrent, ThermoFisher). The designed multiplex PCR primer set then was tested on controlled degraded DNA and challenging human samples - hair, bone, teeth.

The results of this project will serve as a starting point for the development of primer pairs/amplicons that span the entire mitochondrial genome.