B175 Optimization and Validation of Mitochondrial DNA (mtDNA) D-Loop Sequencing on the MiSeq®

Laura A. Wilson, BS, Penn State University, 107 Whitmore Lab, University Park, PA 16802; Sarah Copeland, BS, 300 Farmstead Lane, #8, State College, PA 16803; Gloria Dimick, MS, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; Charity A. Holland, MPH, Mitotyping Technologies, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; Robert Bever, Mitotyping Technologies, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; and Mitchell M. Holland, PhD, Penn State University, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will better understand how transcriptase-adapted Polymerase Chain Reaction (PCR) primers compare to the conventional PCR primers used today by forensic laboratories in the sequencing of the mitochondrial D-loop and the impact this comparison will have on the forensic science community.

This presentation will impact the forensic science community by aiding forensic laboratories in the adoption of a next generation sequencing approach when using the human mitochondrial D-loop protocol from the Illumina® MiSeq® instrument. Through heteroplasmy detection and reporting, sequencing the D-loop with a next generation approach on the MiSeq® will also increase the discrimination power of the testing method.

The mtDNA is present in high copy numbers, making it a powerful tool for analyzing forensic samples such as hair shafts and aged skeletal remains.[1,2] The non-coding region, or D-loop, is the target most often analyzed by forensic laboratories and contains two hypervariable regions known as HVR1 and HVR2; 16,024-16,365 and 73-340, respectively.[3] Sequencing the D-loop with a next generation approach on the MiSeq® will increase the discrimination power of the testing method via heteroplasmy detection and reporting. The D-loop protocol from Illumina® utilizes two sets of conventional, overlapping PCR primer pairs that span the hypervariable regions, with each primer possessing a transposase sequence added to the 5'-end that allows for library preparation prior to analysis on the MiSeq®.[4]

The first-round PCR amplification with Transposase Adapted (TA) primers was optimized and compared to the conventional primer pairs used today by forensic laboratories. The main difference between the two protocols (besides the modified primers) is the use of AmpliTaq® Gold® DNA polymerase versus Ex Taq™ Hot Start from TaKaRa, a polymerase with 3' to 5' exonuclease proofreading activity that utilizes an optimized Ex Taq™ buffer system.[5] The limitations of the PCR reaction parameters were also tested; for example, primer concentrations, magnesium concentration, and the amount of Ex Taq™ employed. The optimized amplification was used in validation studies performed by this group on the Illumina® D-loop protocol following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. The studies included: (1) the evaluation of the robustness of the first-round PCR amplification when using the TA primers or the conventional primer pairs; (2) the sensitivity of library preparation by adding a range of DNA (amplicon) inputs; (3) mixture studies with various ratios of contributor DNA; (4) the evaluation of precision and accuracy through repeatability (same operator and detection instrument); and, (5) concordance experiments. These findings represent an important step toward the adoption of a next generation sequencing approach by forensic laboratories by using the D-loop protocol from Illumina® on the MiSeq® instrument.

Reference(s):

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3. Anderson S., Bankier A.T., Barrell B.G., DeBruijn M.H.L., Coulson A.R., Drouin J., Eperon I.C., Nierlich D.P., Roe B.A., Sanger F., Schreier P.H., Smith A.J.H., Staden R., Young I.G. Sequence and organization of the human mitochondrial genome. *Nature*. 1981. 290:457-65.
4. Human mtDNA D-loop hypervariable region guide. *Illumina*. 2013.
5. *Ex Taq*™ DNA polymerase: a robust PCR enzyme with proofreading activity. *TaKaRa*. 2015.

Next Generation Sequencing, Mitochondrial D-Loop, PCR

*Presenting Author