

USE OF mitoPrimers™ AND CleanAmp MASTER MIX™ FOR MITOCHONDRIAL DNA TESTING

Sabrina Shore, MS¹, Natasha Paul, PhD¹, Gerald F. Zon, PhD¹, Rhonda K. Roby, PhD, MPH²

¹TriLink BioTechnologies, Inc.

²Department of Forensic and Investigative Genetics, UNT Health Science Center

Methods of identifying human biological material are continually improving and expanding: new multiplex STR kits, optimized buffers, high-performing robotics, and sophisticated software. While commercially available kits for amplification of genomic DNA, *i.e.*, STRs, are established and validated by the forensic community, the mitochondrial (mtDNA) reagents and protocols used by different laboratories are more diverse since there are no validated mtDNA kits commercially available. These protocols may employ a variety of DNA polymerases, primers, other PCR accessory reagents and thermal cycling conditions, which all can influence the quality of the downstream data for Sanger dideoxy sequencing. Now, off-the-shelf primers and master mixes are available for mitochondrial DNA testing. mitoPrimers™ (TriLink BioTechnologies, Inc., San Diego, CA) are the same primers the forensic community has been using for over 25 years to interrogate the Control Region of the mitochondrial genome. These primers are HPLC purified and pre-aliquotted into convenient dilute-and-go 100 nmole vials. The primers are vacuum-desiccated, subjected to quality control testing, and available by overnight delivery. Additionally, a PCR master mix, containing CleanAmp™ dNTPs (TriLink BioTechnologies, Inc.), was developed and optimized specifically for the amplification and downstream analysis of the control region of mtDNA. One of the key components in this PCR master mix is a hot start reagent which uses nucleoside triphosphates modified with a thermolabile protecting group. This modification blocks low temperature primer extension and is released at higher temperatures to allow for more specific DNA polymerase incorporation. The goal was to optimize amplification success for samples requiring mtDNA testing by improving PCR yield and specificity, decreasing potential human error from the addition of individual components, and providing reagents that are quality control tested prior to release. The primers, the Master Mix, and the QC testing performed will be presented.