Abstract

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 generic DNA are established and validated by the forensic community, mitochondrial (mtDNA) amplification and sequencing reagents and protocols remain unstandardized. mtDNA amplification and sequencing protocols employ a variety of DNA polymerases, primers, other PCR accessory reagents and thermal cycling conditions, which all can influence the quality of downstream data for Sanger sequencing. Trilink has developed standardized mtDNA analysis reagents. Now, off-the-shelf primers and soon master mixes will be available for mtDNA testing. Trilink’s mitoPrimers™ 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, diute-and-go 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), is being developed and optimized specifically for the amplification and downstream analysis of the control region of mtDNA, the CleanAmp™ mtDNA PCR 2X Master Mix. The key component in this PCR master mix is hot start dNTPs which employ a thermostable 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 is to optimize amplification success 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 development and the QC testing performed will be presented.

Figure 1: Quality Controlled mitoPrimers™ for Amplification and Sequencing of the mtDNA Control Region

Figure 2: mitoPrimers™ Experimental Workflow

Figure 3: Primer Purity is Important for High Quality mtDNA Sanger Sequencing Data

Figure 4: CleanAmp™ Hot Start dNTP Activation Mechanism

Figure 5: CleanAmp™ mtDNA PCR 2X Master Mix Improves Workflow

Figure 7: Developing a CleanAmp™ mtDNA PCR 2X Master Mix that Will Increase Target Yields and Save Time

Conclusion

- mitoPrimers™ undergo a proprietary purification process and are QC validated to ensure high quality Sanger sequencing results.
- The use of CleanAmp™ mtDNA PCR 2X Master Mix streamlines the PCR workflow, thereby reducing the possibility of human error, saving on cost and shortening preparation time.
- mitoPrimers™ are quality control tested with NIST-certified DNA. This allows for their use in the PCR amplification and Sanger sequencing of mtDNA samples.
- Future developments include a formulation which can be employed in fast PCR cycling protocols to achieve high quality data in as little as 30 minutes.

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