A156 Characterization of DNA-Based Certified Reference Materials With New and Emerging Technologies

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After attending this presentation, attendees will understand the principles of digital Polymerase Chain Reaction (PCR), high-throughput sequencing, Sanger sequencing, and fragment-based Short Tandem Repeat (STR) typing for the characterization of DNA-based reference materials used in the forensic DNA typing community.

This presentation will impact the forensic science community by detailing the techniques and protocols used for characterizing National Institute of Standards and Technology (NIST) Standard Reference Materials. The reference materials are commonly used for the calibration of STR typing methods and sequence determination, in addition to quantitative PCR in forensic laboratories.

Over the past 20 years, the Applied Genetics group at the U.S. NIST has been providing DNA-based Standard Reference Materials (SRMs) for the human identity testing community. Current examples include: SRM 2391c for STR typing; SRM 2372 for human DNA quantitation; and SRMs 2392 and 2392-I for mitochondrial sequencing DNA analysis.

SRM 2391c is required by the Federal Bureau of Investigation (FBI) DNA Quality Assurance Standards (Standard 9.5) to calibrate DNA typing procedures performed in forensic laboratories. SRM 2391c consists of genomic DNAs (≈100 ng in 50µL) that have been highly characterized for forensically relevant markers such as core autosomal and Y chromosomal STRs. The characterization of the forensic STR markers of interest is typically a combination of either Sanger sequencing or fragment size analysis. With the upcoming addition of new core autosomal STR loci and supplemental loci contained in commercial Y STR kits, updated information will be added to the characterization of SRM 2391c.

SRM 2372 is typically used for assigning a concentration to a working DNA standard for quantitative PCR measurements. Accurate quantitation of casework DNA extracts is important for determining optimal input amounts for the downstream PCR amplification of STR markers. The three components of SRM 2372 have been certified for Decadic Attenuance (Absorbance) in the single stranded state. This allows the assignment of a working informational value for each component in nanograms per microliter (ng/µL) (by assuming that one OD at 260nm equals 37µg/mL). Moving forward, using digital PCR methods to associate “copy number” characterization to nanograms per microliter concentration may prove even more useful in certain situations.

SRM 2392 and 2392-I contain extracted human DNA that has been characterized for the entire mitochondrial genome (≈16,569 base pairs) by Sanger sequencing methods. Multiple strands of coverage in both the forward and reverse direction across the entire mitochondrial genome ensures confidence in the base calls.

With the emerging application of High-Throughput Sequencing (HTS) and digital PCR technologies to genome characterization, NIST is starting to explore deeper characterization of forensic DNA-based SRMs. The considerations for additional characterization of reference materials include: source of genomic DNAs; amount of DNA required; genetic markers to be typed; analysis by multiple technology platforms; and the specific needs of the forensic community. This presentation will review the past SRMs and identify requirements for the analysis of future forensic reference materials. Examples of HTS and Sanger sequencing of SRMs 2391c and 2392 will be presented along with initial digital PCR results for copy number determination of SRM 2372. These emerging technologies often enable a more accurate assessment of the existing SRMs and provide added value to the forensic community.

PCR, STR, Mitochondrial

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