NIST PCR-BASED DNA PROFILING STANDARD (SRM 2391D): WHAT IS NEW AND DIFFERENT?

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The fifth installment of the NIST Standard Reference Material (SRM) 2391 series was recently released as SRM 2391d: PCR-based DNA Profiling Standard. This set of highly characterized DNA samples comprise the most comprehensive forensic DNA standard of the 2391 series and expands upon the predecessors in terms of certified markers. Four of the five components are extracted DNA derived from buffy coat samples with the fifth being cells spotted onto FTA paper.

The DNA forensic community has progressed greatly in the 24 years since the first release of SRM 2391 in 1995, which was certified initially for variable number tandem repeat (VNTR) and dot-blot hybridization markers and was later updated in 1998 with autosomal short tandem repeat (STR) markers. The subsequent two iterations, 2391a and 2391b, were certified for only autosomal STR markers, but it wasn't until SRM 2391c was released in 2011 and later updated in 2015 and 2018 [1] that other forensically-relevant markers were added to the Certificate of Analysis (COA). These included Y-STR markers, X-STR markers, insertion/deletion (indel) markers, and single nucleotide polymorphism (SNP) markers. The goal when developing SRM 2391d was to provide a highly characterized set of genomic DNA samples for all commercially available forensic DNA markers at the time of certification. This includes of all the markers previously covered in the updated version of SRM 2391c as well as whole mitochondrial genome DNA (mtDNA) sequences, insertion/null allele (INNUL) markers, and Y-SNP markers. High confidence allele calls were established by using multiple polymerase chain reaction (PCR) length-based STR typing kits and sequence-based kits and technologies. Sanger sequencing was still performed for certain markers to resolve questions and characterize regions outside of the commercial testing kits. The range of certified and information values that are associated with this SRM, how these values were assigned [2], as well as the various methods used to obtain these values including capillary electrophoresis (CE) and next generation sequencing (NGS) will be presented.

An additional feature of SRM 2391d is that concentrations of each sample are provided in the COA as an information value, whereas previously it was provided as a range. The concentrations were determined by droplet digital PCR (ddPCR) and are listed with an expanded uncertainty.

Several interesting characterization challenges that arose during the SRM production, such as a large allele from one marker present in the adjacent marker, length-based allele call differing from the sequence-based allele call, and nomenclature differences, will be discussed.

SRM 2391d enables the standardization and calibration of the typing process for STR and other sequence-based markers for human identity testing. Other important uses such as quality control, traceability and validation of new technologies in the forensic community will be highlighted.

References:

[1] SRM 2391c: PCR-Based DNA Profiling Standard Certificate of Analysis (2018). Available online at https://www-s.nist.gov/srmors/certificates/2391c.pdf. Accessed June 27, 2019.
[2] Thompson, A.; Taylor, B.N.; Guide for the Use of the International System of Units (SI); NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/physical-measurement-laboratory/special-publication-811. Accessed June 27, 2019.