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Make it "SNPPY" - Updates to SRM 2391d: PCR-Based DNA Profiling Standard

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ABSTRACT

Standard Reference Material (SRM) 2391d: PCR-Based DNA Profiling Standard was released to the forensic community in 2019. Next Generation Sequencing (NGS) was used as the primary method of certification, where certified values were assigned when a high coverage sequence string was available for a marker. Using NGS to assign values has allowed for additional marker sets beyond short tandem repeat (STR) loci, including single nucleotide polymorphisms (SNPs) and mitochondrial DNA (mtDNA) whole genome sequences, to be included in the Certificate of Analysis (COA). Since the 2019 release, several commercial NGS panels have become available including the Verogen ForenSeq mtDNA Control Region, mtDNA Whole Genome, MainstAY, and Kintelligence Kits. In addition, three community Ion AmpliSeq panels from Thermo Fisher (MH-74 Plex, VISAGE, and Y-SNP) are now available. While the mtDNA whole genome sequence for the components are already included and no new STR markers are introduced by MainstAY, the other recently released panels allow for the inclusion of > 11,000 additional SNPs (e.g., identity, ancestry, phenotype, kinship, and X- and Y-SNPs) and 74 microhaplotypes to the COA for SRM 2391d in an update completed by fall of 2022.

1. Introduction

Technology for sample typing continues to improve within the DNA forensic community. Commercial vendors frequently release new reagent kits and panels for testing to include new marker sets and/or additional markers within an existing set. Once the commercial kits are released, Standard Reference Material (SRM) 2391d: PCR-Based DNA Profiling Standard is tested at the National Institute of Standards and Technology (NIST) with the latest multiplex capillary electrophoresis (CE) commercial kits and sequencing panels to report values for new markers and to determine if there are any genomic differences occurring within these markers. SRM 2391d is typically used in a forensic lab to meet the Federal Bureau of Investigation (FBI) Quality Assurance Standards (Standard 8.4) [1] and for instrument and commercial kit validation studies. This is an important role that the SRM plays in the community, and therefore the components included are well-characterized samples with values for all markers systems that are commercially available and typed in forensic DNA laboratories. Thus, it is critical to perform updates to the Certificate of Analysis (COA) [2] every few years to include the new technology and chemistries released within that time.

2. Materials and methods

SRM 2391d is comprised of five components (A-E). Components A-C, and E are single source samples, while Component D is a 3:1 mixture of Components A and C. CE-based typing was performed with eight additional short tandem repeat (STR) multiplex kits including the NGM SElect Express and GlobalFiler IQC polymerase chain reaction (PCR) Amplification Kits from Thermo Fisher (Waltham, MA) and the Investigator ESSplex SE QS, IDplex Plus, IDplex GO!, 26plex QS, Argus Y-28 QS, and Argus X-12 QS Kits from QIAGEN (Hilden, Germany). Manufacturers' protocols were followed for all the STR-typing CE kits and a 3500xL Genetic Analyzer was used for fragment analysis, except for a validated 15 s injection time with the two Thermo Fisher kits.

Nine next generation sequencing (NGS) marker panels were sequenced for the update to include the ForenSeq MainstAY, Mitochondrial DNA (mtDNA) Whole Genome, mtDNA Control Region, and Kintelligence Kits from Verogen (San Diego, CA), as well as the Precision ID mtDNA Control Region Panel from Thermo Fisher and three

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Table 1
The marker type, number of certified loci, and number of information loci are listed in black font representing the original number of loci in the 2019 release of SRM 2391d: PCR-Based Profiling Standard or red font representing the number of loci available in the 2022 update.

Marker Type	Number of Certified Loci	Number of Information Loci
Autosomal STR	35	13
Y-STR	28	3
X-STR	7	5
Mitochondrial DNA	-	Full mtGenome
Indel/Innuls	-	50
SNPs	-	323 → 11,590
Microhaplotypes	-	74

community sequencing panels (Ion AmpliSeq Microhaplotype (MH)– 74 Plex [3], Ion AmpliSeq VISAGE [4], and Ion AmpliSeq Y-SNP [5] panels). The prototype PowerSeq 46GY from Promega was tested prior to the 2019 release of SRM 2391d; however, PowerSeq 46GY is now commercially available and was retested with SRM 2391d components. Manufacturers' protocols were followed for all the commercially available sequencing panels from Verogen, Thermo Fisher, and Promega. Sequence data was generated on a MiSeq FGx instrument (Verogen and Promega panels) or an Ion S5 XL instrument (Thermo Fisher and Ion AmpliSeq panels). Data analysis of FASTQ files was performed with a custom in-house pipeline based on STRait Razor v3.0, an open-source software [6].

3. Results and discussion

Information gained from testing a total of seventeen additional CE kits and sequencing panels was added to the SRM 2391d: PCR-Based DNA Profiling Standard COA. Most of these commercial kits were made available since the 2019 release of the SRM. The mtDNA whole genome and control region sequences for the components are currently included and no new autosomal-, Y-, or X-STR markers are introduced by the additional CE kits, ForenSeq MainstAY, or PowerSeq 46GY STR sequencing panels. However, other recently released sequencing chemistries including Kintelligence, Ion AmpliSeq VISAGE, Ion AmpliSeq Y-STR, and Ion AmpliSeq MH-74 Plex panels allow for the inclusion of 11,267 SNPs to the 323 SNPs already included (a total of 11,590 SNPs) and 74 microhaplotypes to the COA for SRM 2391d in the 2022 update. The additional SNPs are comprised of six types: identity, ancestry, phenotype, kinship, and X- and Y-SNPs. (Table 1).

4. Conclusions

SRM 2391d: PCR-Based Profiling Standard is one of the most highly characterized forensic DNA standards available. The latest iteration was further characterized to include the marker sets present in commercially or community available panels. Due to continually evolving technology and forensic marker sets, updates to SRM 2391d are necessary every 2 to 3 years to capture recently released chemistries. Because sequencing is the primary method for certification for SRM 2391d, this allows additional marker sets, such as SNPs, mtDNA whole genome and control regions, and MHs to be associated and included in the COA. While there were 323 SNPs listed in the original certificate, the update includes a total of 11,590 SNPs and 74 microhaplotypes in the 2022 update.

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Conflict of interest

None.

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All work performed at NIST has been reviewed and approved by the National Institute of Standards and Technology Research Protections Office. This study was determined to be "not human subjects research" (often referred to as research not involving human subjects) as defined in U. S. Department of Commerce Regulations, 15 CFR 27, also known as the Common Rule (45 CFR 46, Subpart A), for the Protection of Human Subjects by the NIST Human Research Protections Office and therefore not subject to oversight by the NIST Institutional Review Board.

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