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# Establishing traceability to NIST SRM 2391c: PCR-Based DNA Profiling Standard

Carolyn R. Steffen*, Margaret C. Kline, David L. Duewer, Peter M. Vallone

*U.S National Institute of Standards and Technology, NIST 100 Bureau Drive, Gaithersburg, MD 20899-8314, USA*

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ABSTRACT

The NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling Standard was updated in April 2015 to contain new information relevant to the forensic community. Previously, there were certified genotypes for 24 autosomal STR markers plus Amelogenin and 17 Y-STR markers. Due to the increase in markers present in larger commercial autosomal STR and Y-STR multiplex kits recently released, there is a need to add certified types for these new markers for each component of SRM 2391c (Components A–F). The updated Certificate of Analysis has certified values for 1 additional autosomal STR marker (D6S1043) and 12 additional Y-STR markers (29 total). Also, the number of STR multiplex assays tested increased from 24 to 43. Sanger sequencing was performed on Components A–C, E and F (Component D is a mixture of Components A and C) for all the certified markers to determine the STR repeat motifs and to characterize adjacent flanking regions and underlying polymorphisms (sequence, insertion-deletion, variation in complex motifs) typically not detected by fragment-based typing. SRM 2391c Components A-F can be used to establish traceability in a laboratory based on the updated certified and reference genotypes/haplotypes (information values cannot be used for NIST traceable materials). The simple steps to achieve NIST traceability with SRM 2391c will be explained.

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## 1. Introduction

The NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling standard is an important tool in the forensic DNA community and was recently updated to contain new information. The updated Certificate of Analysis (COA) contains certified values for one additional autosomal STR marker and 12 additional Y-STR markers from the original document that was released in 2011. Sanger sequencing was performed on Components A–C, E and F (D is a mixture of Components A and C) for all the certified markers (25 autosomal STR markers plus Amelogenin and 29 Y-STR markers) to verify and confirm the genotypes/haplotypes obtained from STR typing [1].

The FBI has adopted the use of SRM 2391c in their Quality Assurance Standards (QAS) under Section 9 on analytical procedures. Specifically, the FBI DNA QAS 9.5.5 states, "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard" [2–4]. Therefore, SRM 2391c is also intended for use to assign values to in-house control materials to ultimately create NIST traceable materials based on the previous and updated certified and reference genotypes/haplotypes (information values cannot be used to create NIST traceable materials). Traceability requires the establishment of an unbroken chain of comparisons to stated references [5]. In the case of DNA testing with autosomal short tandem repeat (STR) markers, the reference material is SRM 2391c. The materials deemed traceable to NIST-created SRMs must have a record associated with them. There are three simple steps necessary to establish traceability to NIST SRM 2391c that will be further described.

## 2. Materials and methods

SRM 2391c is comprised of six components (A–F). It is recommended that all six components are used to establish traceability, but it is possible to use any one of the components for this process. The starting material can be a well-characterized in-house DNA sample of appropriate concentration and volume. Alternatively, there are several commercial sources of "control" DNA samples that may also be fit for purpose (i.e. 2800 M, 9947A, 9948, 007). SRM 2391c is updated to have certified and reference values for STR markers that are present in commercial kits (e.g. Globalfiler, PowerPlex Fusion). Therefore, most commercially

* Corresponding author. Tel.: +1 301 975 4275; fax: +1 301 975 8505.
*E-mail address:* becky.steffen@nist.gov (C.R. Steffen).

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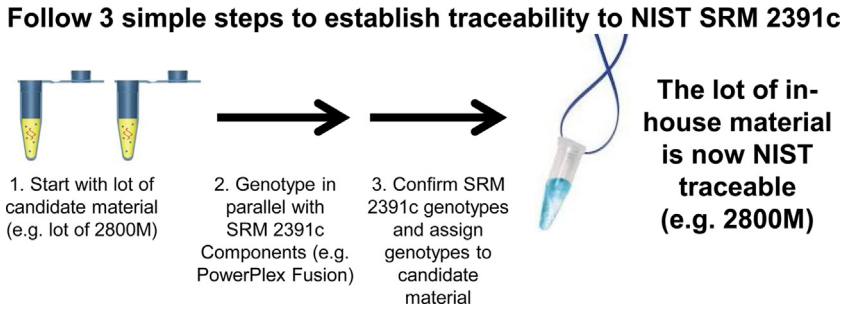


**Fig. 1.** Illustrates the 3 simple steps to establish traceability to NIST SRM 2391c with 2800 M and PowerPlex Fusion as an example.

available STR multiplex kits can be used to establish traceability with the SRM 2391c. To highlight an example of this process, a single lot of 2800M (Promega, Madison, WI) at a concentration of 10 ng/μL in a total volume of 25 μL was used as the candidate DNA material. 2800 M and Components A–F of SRM 2391c (the paper substrate Components E and F were previously extracted) were diluted to 0.5 ng/μL in deionized water. One microliter of each sample was typed with full reactions of PowerPlex Fusion (Promega) according to manufacturer's protocol using a 9700 thermal cycler (Thermo Fisher, Carlsbad, CA). A 3500xl Genetic Analyzer with POP-4, 36 cm array (Thermo Fisher) was used for separation and detection of the PCR amplicons (injection parameters were 1.2 kV for 15 s). Data analysis and interpretation were performed with GeneMapper ID-X v1.4 (Thermo Fisher).

## 3. Results and discussion

Fig. 1 illustrates the three steps necessary to establish traceability to SRM 2391c. Step 1 is to choose the lot of candidate material intended to become NIST traceable (e.g. 2800 M). It is responsibility of the laboratory to ensure that the DNA solution is homogeneous and stable under storage conditions. Step 2 is to genotype the in-house material in parallel with the six SRM 2391c components using the STR multiplex kit of choice (e.g. PowerPlex Fusion). Step 3 is to first confirm the certified and reference genotypes of the six components of SRM 2391c and then assign genotypes to the lot of candidate material. Once all three steps have been completed, the in-house material is now considered NIST traceable (e.g. the lot of 2800 M is now a NIST traceable material). It is important to keep all documentation of this process and have a saved record associated with each lot of NIST traceable material. If a new lot of candidate material is to be made traceable the process must be repeated (even if the new lot is from the same source).

## 4. Conclusions

With the recent update of SRM 2391c, there is new information relevant to the forensic community available. As of April 2015, all of the commercially available autosomal STR and Y-STR markers have certified genotypes/haplotypes associated with them for the six components of SRM 2391c. The previous and new certified values, as well as the reference values for additional autosomal STR markers can be used to make in-house control material NIST traceable with three simple steps. Many laboratories do not take full advantage of this process because there is a perception that it is a long, arduous procedure when in effect it is a simple process that requires little time and documentation to complete. The importance of having NIST traceable material available for instrument and kit validations, as well as performance and quality control checks, is invaluable to the forensic DNA typing process.

## Conflict of interest

None.

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