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TECHNICAL NOTE

Criminalistics

Development of a forensic DNA research grade test material

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Abstract

Advancements in forensic DNA typing technology and methods have increased sensitivity and, while beneficial, carry the weight of more challenging profile interpretation. In response, the forensic DNA community has often requested more complex reference materials to address commonly encountered measurement and interpretation issues such as complex DNA mixtures, DNA degradation, and PCR inhibition. The National Institute of Standards and Technology (NIST) released Research Grade Test Material 10235: Forensic DNA Typing Resource Samples to support the forensic DNA community. Components include three single source samples, two degraded samples, and three mixture samples. As part of the Research Grade Test Material (RGTM) process, automated methods for bottling, alternative sample tube types, and the addition of carrier RNA for stabilizing low-quantity samples were investigated. Both internal and external testing demonstrate the stability of the material over time at 4°C through qPCR testing. In the development of a data portal, users have been allowed to anonymously upload results and compare their data with NIST and others. This report describes the preparation and stability of this material.

KEYWORDS
forensic DNA, PCR, quantification, reference materials, STR, training samples, validation

Highlights

- Forensic DNA Research Grade Testing Material developed to assist in validation and training efforts.
- Initial data indicates that the material is stable at 4°C at approximately 5 ng/μL.
- Data-sharing portal was built to accept community-generated data.

1 | INTRODUCTION

The evolution of forensic DNA typing technologies has significantly enhanced sensitivity, leading to more complex short tandem repeat (STR) profile interpretation. Forensic DNA testing laboratories often encounter samples with low template quantity, degradation, and DNA from multiple contributors. These challenges can make estimating the number of contributors in a mixed sample, achieving clear peak resolution, and making accurate allele calls difficult. In response, the forensic DNA community has requested more complex reference materials that can provide ground truth or certified information on these properties (quantity, allele calls, number of contributors, etc.). However, due to

the inherent variability of these sample types, achieving robust characterization and ensuring long-term stability for a Standard Reference Material (SRM) classification by the National Institute of Standards and Technology (NIST) remains a challenge.

To address these sample preparation and characterization challenges, the NIST Office of Reference Materials has introduced a new classification of exploratory material called a Research Grade Test Material (RGTM). RGTMs aim to assess fit-for-purpose requirements collaboratively, enabling a more effective approach to complex measurement challenges [1]. Data from RGTMs is generated collaboratively with users to determine relevant values for what is being measured and the long-term stability of the material. For example, data may be generated to support the long-term stability of a material, consensus of a measurement value, or to assess if a material is fit-for-purpose.

On August 31, 2023, RGTM 10235: Forensic DNA Typing Resource Samples was released to the forensic DNA community. RGTM 10235 is composed of eight well-quantified DNA extracts. Samples include three single-source samples, two degraded samples, and three mixture samples.

RGTM 10235 provides a more complex standardized set of samples that may assist forensic DNA laboratories in:

- *Validation of new methods*: In conformance with laboratory accreditation and the Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) [2], forensic DNA laboratories are required to validate instrumentation, methods, and software used in their operational settings. Additionally, public calls have been made for collaborative validation efforts and standardized sample sets in the community [3, 4]. RGTM 10235 offers a standardized set of samples to assist in harmonizing these measurements and allows laboratories to compare their results with other laboratories collaboratively.
- *Training staff*: The standardized samples and publicly available data on the STRBase website [5] are valuable tools for training new staff and improving existing skills through ongoing monitoring of results. Additionally, training and performance checks are required under the QAS [2] and using RGTM 10235 may assist laboratories by providing a standardized set of samples to compare results within a laboratory and across the community.
- *Collaborative data sharing*: Laboratories often use internally collected samples for training and validation, raising concerns about data shareability beyond individual labs. Within the community, there is ongoing concern about sample origin, consent, and data sharing in forensic DNA research and validation [6–9]. RGTM 10235 samples were acquired from a commercial blood bank with explicit informed consent for publicly sharing samples and data [10]. While the samples are permitted to be shared publicly, they are not to be searched in databases and are not permitted for whole genome sequencing.

The RGTM program fosters collaboration by providing materials to laboratories in exchange for sharing data collected from the samples. The data collected will inform NIST on new automated filling methods, tube types, and sample additives for future research and Reference Material production. This data will also ensure that production changes don't compromise downstream measurements in forensic laboratories. Measurements collected by users of the RGTM Samples are anonymously submitted and collaboratively shared through the STRBase data portal, then curated by NIST for public sharing. For example, quantification measurements will support the confirmation of the materials' long-term stability. The same information may allow a user to benchmark their quantification method. It should be noted that the measurement values of the RGTM are not certified by NIST as with a traditional Standard Reference Material (SRM). The RGTM may be thought of as a common set of test materials for the community to utilize. The information gained from this collaborative effort will educate the forensic community about measurement variations and aid in developing future materials with reduced measurement uncertainty.

Ultimately, RGTM 10235 allows the community to publicly share data generated, results of methods examined, and genetic profiles due to their informed consent, and the data generated will assist in the development of future reference materials produced by NIST for the forensic DNA community.

2 | MATERIALS AND METHODS

2.1 | DNA samples

RGTM 10235 is composed of eight components labeled Samples 1–8. All samples are human genomic DNA extracts from whole blood derived from single-source anonymous donors (iSPECIMEN, Lexington, MA) under the approval of the NIST Research Protections Office. The samples within RGTM 10235 were purchased under informed consent, which explicitly allows for sharing genetic data pertaining to STR analysis with collaborators, researchers, and law enforcement agencies such as crime laboratories [10]. Donors consisted of three female and five male donors. Each unit of donated whole blood (~500 mL each) was aliquoted into 50 mL conical (50 mL per aliquot) and stored at −80°C until extraction.

2.2 | DNA extraction

Blood was thawed and aliquoted (5 mL per aliquot) into sterile 50 mL conical tubes prior to extraction. A modified salt-out manual extraction protocol [11] was performed for each aliquot. All extraction chemicals used to prepare buffers were purchased from Sigma Aldrich (St. Louis, MO). Extraction consisted of adding 30 mL Red Blood Cell Lysis Buffer (144 mmol/L ammonium chloride, 1 mmol/L sodium bicarbonate in deionized water) to the 5 mL of blood. Samples were mixed by gentle inversion and incubated at room temperature for 15 min. After incubation, samples were centrifuged at 2000 rpm for 20 min. The supernatant was discarded, and 9 mL of Nucleic Lysis Buffer (10 mmol/L

Tris–HCl, 400 mmol/L sodium chloride, 2.0 mmol/L disodium ethylenediaminetetraacetic acid in deionized water) was added to resuspend the pellet. Buffers containing 300 µL of 20% sodium dodecyl sulfate and 1.8 mL of Protease K Solution: 5 mg Protease K per 1 mL Protease K Buffer (2.0 mmol/L disodium ethylenediaminetetraacetic acid, 1.0% (mass per volume) sodium dodecyl sulfate in deionized water) were added and mixed by inversion, followed then by incubation in a shaking water bath at 37°C overnight.

After overnight incubation, 4 mL saturated ammonium acetate solution (in deionized water) was added, and samples were shaken vigorously for 15 seconds. Samples were centrifuged at 4500 rpm for 10 min. The supernatant was transferred to a new sterile 50 mL conical tube, and 30 mL absolute ethanol was added. Tubes were then mixed by gentle inversion until the DNA aggregated and floated to the top of the tube. A disposable inoculating loop (Thermo Fisher Scientific, Waltham, MA) was used to spool the DNA and transfer it to a sterile 15 mL conical tube to air dry.

The DNA pellet was air-dried overnight and rehydrated in 2 mL TE^{-4} buffer (Thermo Fisher Scientific) increments until fully solubilized. Each sample was produced from 350 mL to 450 mL of solubilized DNA and was stored at 4°C in sterile perfluoroalkoxy fluoropolymer (PFA) previously sterilized containers (Savillex, Eden Prairie, MN) until preparation for production.

2.3 | Digital PCR

Bulk sample concentrations were determined through digital PCR (dPCR) using a Bio-Rad QX200 Droplet Digital PCR System (Hercules, CA). The previously developed and validated NEIF assay (targeting the EIF5B gene on chromosome 2) dPCR reaction setup thermal cycling parameters, as reported in Romsos et al. [12], were used for quantification. The assay consists of a forward primer (gccaaacttcagccttctcttc, Eurofins, Louisville, KY), reverse primer (ctctggcaacatttcacactaca, Eurofins), and FAM labeled BHQ+ probe (tcatgcagttgtcagaagctg, LGC Biosearch Technologies, Novato, CA). The NEIF assay was determined to be a single copy target of 67 base pairs in length. These results were exported to an Excel spreadsheet for further manipulation.

The digital PCR setup consisted of 12.5 µL BioRad Supermix for Probes (no dUTPs), 1.9 µL Forward Primer (5 µmol/L), 1.9 µL Reverse Primer (5 µmol/L), 1.2 µL BHQ+ FAM-labeled Probe (5 µmol/L), 5.0 µL PCR grade water, and 2.5 µL of original sample diluted 1:10. Each sample was run in quadruplicate.

For all digital PCR experiments, droplets were generated on the Auto Droplet Generator using the Droplet Generation Oil for Probes (BioRad). Droplets were thermal cycled on a ProFlex thermal cycler (Thermo Fisher Scientific) for 95°C for 10 min followed by 60 cycles of 94°C for 0.5 min and 60°C for 1 min, then 98°C for 10 min before a 4°C hold until the plate was removed. Droplets were read on the QX200 Droplet Reader and analyzed using the QuantaSoft Analysis Software version 1.7.4.0917. The number of positive and negative droplets at the end of 60 cycles was determined by visually setting assay-specific intensity thresholds for each run. Data was exported into Excel for further analysis. Original extracted stock concentrations were determined by converting the calculated lambda value to nanograms per microliter and accounting for the original 1:10 dilution [13].

2.4 | Sample preparation and bottling

Single source samples (Samples 1 through 5) were diluted from the bulk material to approximately 5 ng/µL in TE^{-4}. Sample 1, Sample 2, and Sample 5 were diluted in a TE^{-4} buffer containing 50 ng/µL of yeast tRNA (Thermo Fisher Scientific) to improve nucleic acid stability. This buffer was prepared by adding 2.5 mL of 10 mg/mL yeast tRNA to 497.5 mL of TE^{-4} buffer for a final concentration of 50 ng/µL. Samples 3 and 4 were diluted in TE^{-4} without adding yeast tRNA.

Sample 2 and Sample 4 were degraded from stocks of Sample 1 and Sample 3, respectively and treated with UV light to simulate degraded casework-type samples. 20 mL aliquots of the 5 ng/µL stock solution were transferred into multiple 30 mL PFA containers placed approximately 5 cm from a 15-Watt short-wave UV light and treated for 5 min within a HEPA-filtered PCR hood. The degraded DNA aliquots were pooled per sample in a larger PFA container to prepare for bottling.

Sample 6, Sample 7, and Sample 8 are mixture samples of donors not found in Samples 1 through 5. The mixture samples were diluted in the yeast tRNA TE^{-4} buffer to a final concentration of approximately 5 ng/µL. Sample 6 is a two-person mixture (Female (F):Male (M)) in a 90:10 ratio. Samples 7 and 8 are three-person mixtures. Sample 7 (F:M:M) is a 20:20:60 ratio, whereas Sample 8 (F:M:M) is a 10:30:60 ratio. RGTM 10235 Sample format and accompanying information are found in Table 1. The letters (A through H) in Table 1 under the Format column denote individual donors in the Samples.

All Samples were prepared in a final volume of approximately 135 mL for bottling in polypropylene screwcap tubes (Sarstedt, Numbrecht, Germany). Final concentrations for all Samples (1 through 8) were confirmed with the dPCR measurement and the NEIF assay. Bottling of the material was performed robotically using a Scinomix VXL automated liquid handling robot (Scinomix, Earth City, MO). Batches of 600 vials of each component were filled with approximately 200 µL per vial. Each vial was transferred to a numbered box in the order they were filled and stored at 4°C.

2.5 | DNA concentration, homogeneity, and stability measurements

Final DNA concentrations were measured with dPCR to assess homogeneity. Two assays (NEIF and ND14) were used for quantification [12]. One vial per box ($n = 6$) was randomly selected for all samples. Three technical dPCR replicates were performed for each vial with both the NEIF assay and ND14 assay.

Additional quantitative PCR (qPCR) measurements were made on a 7500 Real-Time PCR System for Human Identification (Thermo Fisher

TABLE 1 RGTM 10235: Forensic DNA resource sample overview.

Sample	Format	Sex	Carrier[a]
Sample 1	Single source A	Male	Yes
Sample 2	Single source A – degraded[b]	Male	Yes
Sample 3	Single source B	Female	No
Sample 4	Single source B – degraded[b]	Female	No
Sample 5	Single source C	Male	Yes
Sample 6	2p mixture D + E	Female:Male – 90:10	Yes
Sample 7	3p mixture D + F + G	Female:Male:Male – 20:20:60	Yes
Sample 8	3p mixture H + G + E	Female:Male:Male – 10:30:60	Yes

[a]Samples with carrier contain 50 ng/μL of yeast tRNA to improve nucleic acid stability.
[b]Degraded with UV light for 5 min.

Scientific) instrument. Data was analyzed using the HID Real-Time PCR Analysis Software version 1.3.1. The following commercial chemistries were run using manufacturer's protocols for the 7500: Quanitfiler Trio (Thermo Fisher Scientific), Quantifiler HP (Thermo Fisher Scientific), PowerQuant (Promega, Madison, WI) and Quanitplex Pro (QIAGEN, Hilden, Germany). The NIST Human DNA Quantitation Standard Reference Material (SRM 2372a) Component A was used to generate an 8-point standard curve (ranging from 49.8 ng/μL to 0.06 ng/μL) for all qPCR assays in triplicate. qPCR measurements were made by testing six tubes in triplicate for all samples of RGTM 10235. Data was exported to Excel for further manipulation.

For stability assessment measurement, the Quantifier Trio qPCR testing results at the time of bottling were compared to values measured after 3 months of storage at 4°C and with the first external confirmation testing data point.

2.6 | Capillary electrophoresis STR testing

Genotyping through capillary electrophoresis (CE) was performed with three commercial autosomal kits: PowerPlex Fusion 6C (Promega), GlobalFiler Express (Thermo Fisher Scientific), and Investigator 24plex (QIAGEN) and three commercial Y-STR kits: Powerplex Y23 (Promega), YFiler + (Thermo Fisher Scientific), and Investigator Argus Y-28 QS (QIAGEN). Samples were amplified following manufacturers' protocols on a ProFlex PCR System (Thermo Fisher Scientific) thermal cycler. CE typing was performed on a 24-capillary Applied Biosystems Prism 3500xL Genetic Analyzer following the manufacturer's recommended protocols for all kits, except for using a 15 s injection time. Data was analyzed using GeneMapper ID-X Software v1.7 (Thermo Fisher Scientific) with a 50 RFU analytical threshold for all samples.

2.7 | External confirmation testing (ECT)

One unit of RGTM 10235 was supplied to Bode Technology Group Inc. (BTG) (Lorton, VA) for external confirmation testing prior to and after the RGTM was made publicly available. DNA extracts were quantified using Quantifiler Trio (Thermo Fisher Scientific) in 11 μL reaction volumes consisting of 5 μL Quantifiler Trio PCR Reaction Mix, 4 μL Quantifiler Trio Primer Mix, and 2 μL DNA and run on an ABI 7500 Real-Time PCR System with HID Real-Time PCR Software version 1.3.1.

STR genotyping was performed at BTG using a 9700 PCR system thermal cycler (Thermo Fisher Scientific) for amplification with the GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific) in 25 μL reaction volumes with a targeted template DNA quantity of 1 ng for 29 cycles. Capillary electrophoresis was performed on a 3500xL Genetic Analyzer using 1 μL of amplification product, POP-4 polymer, and 3500xL Data Collection software version 4.0.1. The injection time was 29 seconds with an injection voltage of 1.2 kV. Raw data were analyzed using GeneMapper ID-X v1.6 with an analytical threshold of 125 RFU and a stochastic threshold of 600 RFU.

3 | RESULTS AND DISCUSSION

3.1 | Concentration of samples

Table 2 summarizes the concentration measurements for each sample in RGTM 10235 performed by dPCR. All sample concentrations were measured between 4 ng/μL and 5 ng/μL as determined by two dPCR assays, except for the two degraded samples (Sample 2 and Sample 4). The degraded samples were measured to be approximately 3 ng/μL. It should be noted that the degraded samples originated from the same stock as their intact counterparts, and their lower concentrations reflect the impact of UV damage on the quantification methods. The concentration reported for RGTM 10235 in the Guidance Document (available with the material from the NIST Store) is from the July 2023 dPCR measurement which encompassed the three technical replicates from one vial per box ($n = 6$).

3.2 | Stability and degradation indices

Examination of the qPCR stability data indicated that each Sample was stable over time, as tested from July 2023 to May 2024. Table 2

TABLE 2 DNA concentration as measured by dPCR and the small autosomal target in the Quantifiler Trio assay.

				dPCR		Quantifiler trio	
Sample	**Measurement date**	**Laboratory**	**Replicates (*n*)**	**Ng/μL**	**SD (ng/μL)**	**Ng/μL**	**SD (ng/μL)**
Sample 1	July 2023	NIST	18	5.1	0.4	4.5	0.4
	October 2023		3	5.0	0.1	4.8	0.1
	April 2024		3	5.2	0.2	5.3	0.2
	July 2023	ECT	1			5.4	[a]
	November 2023					**5.6**	
	May 2024					**5.8**	
Sample 2	July 2023	NIST	18	3.6	0.6	**2.1**	0.4
	October 2023		3	3.5	0.6	**2.4**	0.2
	April 2024		3	3.4	0.5	3.1	0.6
	July 2023	ECT	1			**2.8**	[a]
	November 2023					**2.6**	
	May 2024					**2.7**	
Sample 3	July 2023	NIST	18	4.9	0.3	4.1	0.6
	October 2023		3	4.7	0.1	**4.0**	0.4
	April 2024		3	4.8	0.1	4.4	0.2
	July 2023	ECT	1			5.1	[a]
	November 2023					5.3	
	May 2024					4.8	
Sample 4	July 2023	NIST	18	3.0	0.6	**1.3**	0.1
	October 2023		3	2.8	0.7	**1.4**	0.1
	April 2024		3	2.9	0.7	**1.6**	0.3
	July 2023	ECT	1			**1.5**	[a]
	November 2023					**1.6**	
	May 2024					**1.6**	
Sample 5	July 2023	NIST	18	5.1	0.4	4.3	0.5
	October 2023		3	5.1	0.2	4.0	0.7
	April 2024		3	5.3	0.1	**5.9**	0.3
	July 2023	ECT	1			**5.7**	[a]
	November 2023					5.2	
	May 2024					5.3	
Sample 6	July 2023	NIST	18	4.5	0.3	4.0	0.7
	October 2023		3	4.4	0.1	**3.5**	0.5
	April 2024		3	4.5	0.1	5.1	0.3
	July 2023	ECT	1			4.6	[a]
	November 2023					**5.2**	
	May 2024					4.2	
Sample 7	July 2023	NIST	18	4.3	0.3	4.2	0.8
	October 2023		3	4.3	0.1	3.9	0.2
	April 2024		3	4.3	0.1	**4.8**	0.1
	July 2023	ECT	1			**5.1**	[a]
	November 2023					**5.0**	
	May 2024					4.3	

(Continues)

TABLE 2 (Continued)

Sample	Measurement date	Laboratory	Replicates (*n*)	dPCR		Quantifiler trio	
				Ng/μL	SD (ng/μL)	Ng/μL	SD (ng/μL)
Sample 8	July 2023	NIST	18	4.3	0.5	4.1	0.3
	October 2023		3	4.2	0.1	4.0	0.1
	April 2024		3	4.2	0.2	4.7	0.0
	July 2023	ECT	1			3.9	[a]
	November 2023					4.6	
	May 2024					3.4	

Note: The bold values represent occurrences where the qPCR measurements fall outside of the original dPCR measurement and uncertainty.
[a]Quantification performed through ECT was tested only once and had no standard deviation.

TABLE 3 Degradation Index value calculated from stability data (qPCR data).

Sample	NIST July 2023	ECT July 2023	NIST October 2023	ECT November 2023	NIST April 2024	ECT May 2024
Sample 1	0.70	0.85	0.71	0.91	0.74	0.95
Sample 2	2.94	4.13	2.88	3.75	2.97	4.01
Sample 3	0.66	0.86	0.65	0.90	0.69	0.87
Sample 4	5.91	7.46	5.58	7.05	6.34	7.28
Sample 5	0.66	0.87	0.62	0.86	0.76	0.88
Sample 6	0.75	0.96	0.68	1.04	0.87	0.91
Sample 7	0.86	1.06	0.80	1.02	0.91	0.96
Sample 8	0.86	0.86	0.82	1.00	0.87	0.77

Note: Samples 2 and 4 are degraded.

shows the stability measurements for each Sample measured at NIST (by dPCR and qPCR) and through ECT (qPCR only). It should be noted that there are occurrences where the qPCR measurements fall outside of the original dPCR measurement and uncertainty (denoted in bold in Table 2). It is hypothesized that due to the size of the amplicon (67 bp for dPCR, 80 bp for Quantifiler Trio small amplicon) there is an overestimation for degraded samples with dPCR, as all but a single qPCR measurement fell outside the original dPCR measurement. Non-degraded samples outside of the original dPCR measurements via qPCR may be due to the relative nature of qPCR being dependent on a standard curve, amplicon size, and differing assay conditions. The average deviation from the dPCR results for the small Quantifiler amplicon in the qPCR measurements is 0.25 ng/μL ± 0.15 ng/μL (range 0.05 ng/μL to 0.52 ng/μL).

An additional result of the qPCR measurement is the degradation evaluation provided by the degradation index in Quantifiler Trio. An index value of 1 or less indicates that the samples are not likely to be degraded [14]. As the degradation index increases, the likelihood of the samples demonstrating degradation patterns ("ski slope" effect, dropout at higher molecular weight loci, etc.) increases. Samples 2 and 4 have a higher degradation index due to treatment with UV light than their non-degraded counterpart (Samples 1 and 3, respectively). As observed in Table 3, the degradation index appears stable over time, suggesting that the material is stable.

3.3 | STR genotyping

In addition to quantitative measurements, STR genotyping was performed for the RGTM 10235 samples with three commercially available autosomal STR and Y-STR kits. Samples were concordant between commercial chemistries, and the degraded samples demonstrated an expected "ski-slope" effect and lower peak heights at higher molecular weight loci. A subset of PowerPlex Fusion 6C electropherograms is shown in Figure 1. The three-person mixture Samples 7 and 8 contain alleles requiring single-nucleotide resolution (D12S391: 17.3 and 18 and D1S1656: 14.3 and 15). Electropherograms for all commercial autosomal STR and Y-STR kit profiles are found in Appendix S1, whereas allele calls are found in Appendix S2. The .hid files generated can be found on the NIST Public Data Repository (https://doi.org/10.18434/mds2-3439) [15].

4 | CONCLUSION

RGTM 10235: Forensic DNA Resources Samples provides an 8-sample set of DNA extracts to forensic DNA laboratories with informed consent allowing for public sharing of the samples and data generated. RGTM 10235 includes three single source samples, two

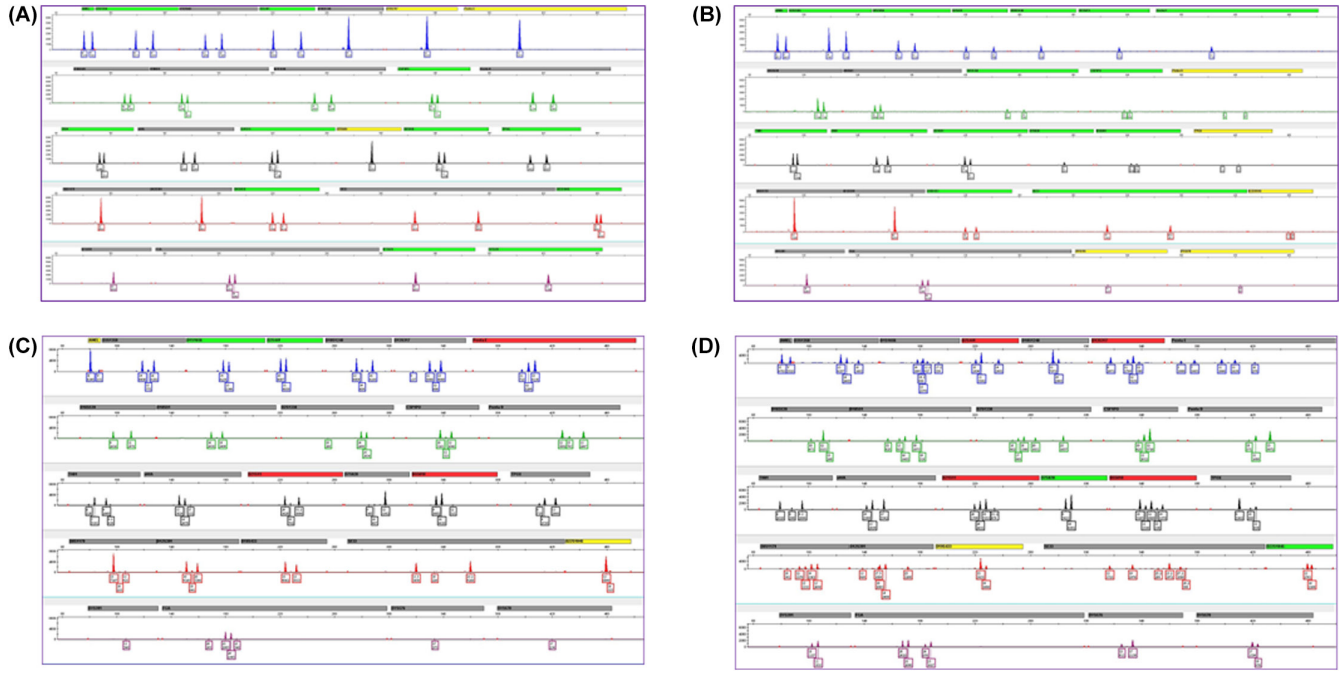

FIGURE 1 PowerPlex Fusion 6C electropherograms for four samples from RGTM 10235: (A) Sample 1 – Single source male; (B) Sample 2 – degraded single source male; (C) Sample 6–2 person mixture in a 90:10 ratio; (D) Sample 8–3 person mixture in a 60:30:10 ratio.

degraded samples, and three mixture samples. In developing this material, the Applied Genetics Group at NIST has created a data portal through the STRBase website to accept practitioner data with an upload mechanism that promotes anonymity to users. This provides the community with a uniform sample set that can be used in forensic DNA laboratories and compared anonymously to other practitioner datasets. The data generated from RGTM 10235 has shown that the material is stable and reliable for forensic validation studies and training exercises. Additionally, RGTM 10235 provides longitudinal data for NIST to better develop future reference material production to keep current with the changing needs of the forensic DNA community.

The goal of providing this material to the forensic DNA community is to offer a set of known samples that may be used to assess new technology and supplement a laboratory's internal validation and training programs. By providing these samples, the ability for laboratories to cross-compare their results to those generated and submitted throughout the community allows laboratories to visualize their results in an informal, ongoing interlaboratory study modality. RGTM 10235 also allows for identifying training gaps within a laboratory by using these samples to generate results across analysts for an intralaboratory variation perspective. Using these samples may benefit the community beyond those listed above by offering known samples for further academic studies, making it possible to test other marker systems, or generating data from interlaboratory studies.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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