
Development of a new (and free) DNA resource to support the forensic DNA typing community
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ISHI
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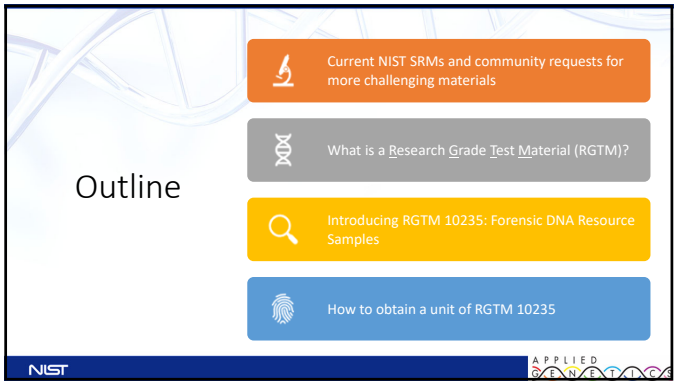
Outline
Current NIST SRMs and community requests for more challenging materials
What is a Research Grade Test Material (RGTM)?
Introducing RGTM 10235: Forensic DNA Resource Samples
How to obtain a unit of RGTM 10235
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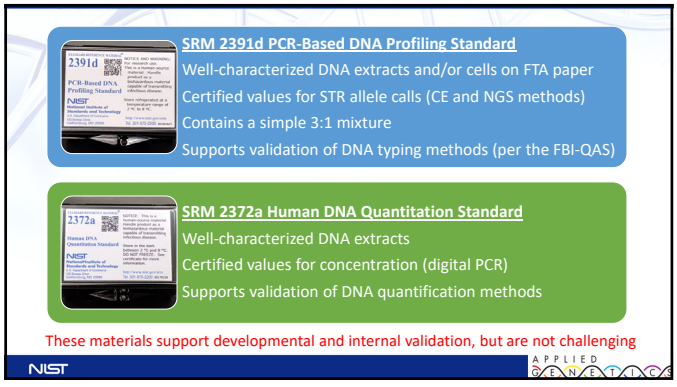
SRM 2391d PCR-Based DNA Profiling Standard
Well-characterized DNA extracts and/or cells on FTA paper
Certified values for STR allele calls (CE and NGS methods)
Contains a simple 3:1 mixture
Supports validation of DNA typing methods (per the FBI-QAS)
SRM 2372a Human DNA Quantitation Standard
Well-characterized DNA extracts
Certified values for concentration (digital PCR)
Supports validation of DNA quantification methods
These materials support developmental and internal validation, but are not challenging
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Community input: Could NIST make an SRM for…?

- Degraded DNA
- Low template DNA
- Complex DNA mixtures
- DNA extracts containing PCR inhibitors
- Rapid DNA methods (swabs)

Standard Reference Materials

Challenges to producing a NIST SRM for these sample types

Degraded and low template samples pose challenges in terms of long-term stability and reproducing the same material over time

- What method should be used to degrade the DNA and to what degree?
- What PCR inhibitors and concentrations should be used?
- How many mixtures, contributors, ratios, etc.?

In general, SRMs aren't typically meant to be 'test' materials

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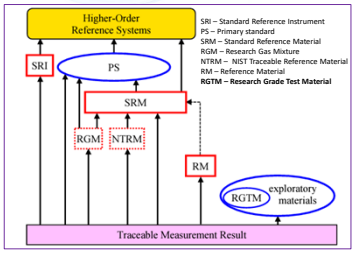
Research Grade Test Material

Introduced in 2020

NIST's Research Grade Test Materials (RGTMs) are a subset of the "exploratory materials" that NIST investigates for use in studying new measurement challenges.

Also coincided with the pandemic



https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf
https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf

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An example of one of the first NIST RGTMs

RGTM 10169

RGTM 10169 SARS-CoV-2 Synthetic RNA Fragments

- In the spring of 2020, we developed a material to support SARS-CoV-2 testing
- Produced and released 90 days
- In vitro transcripts (IVTs) RNA SARS-CoV-2
- Characterized by digital PCR (RT-dPCR) for copies/µL
  - **But not certified values (i.e. SI traceable)**
- Support benchmarking and validation of SARS-CoV-2 Dx
- **Minimal homogeneity and stability testing**
- Distributed for free
- 320 Units/32 countries/173 labs
- **Address an immediate measurement challenge**

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Produce a material for the forensic community

NIST can explore new ideas on material production and provide more challenging samples to the community

Users can obtain the samples for free and provide data to support stability, quality, and utility (***to determine if these would be fit for purpose***)

Users = forensic labs, commercial testing labs, academia, vendors

RGTM Purpose

Assist in internal validation efforts by providing a common supplemental sample set

Used as training samples within a laboratory

Used in research studies (peer-reviewed publications)

Informed consent allows for sharing of samples and data

Inform future SRMs and RMs provided to the forensic DNA community

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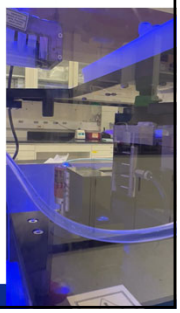
New ideas/methods introduced in the RGTM

- Alternative tubes for storage (transitioning away from Teflon/PFA tubes)
- The addition of a 'carrier' (yeast tRNA) to stabilize lower-concentration samples
- Automated filling, capping, and labeling
- Providing larger volumes (200 µL vs 55 µL)
- UV-degraded samples
- 2- and 3-person mixtures
- Subjected to limited stability testing



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Informed Consent

- Desire for an explicit informed consent for donors
- Outlines primary intent to **share samples with law enforcement (LE) laboratories**
- Allows for the **public sharing of genetic information** (e.g. STRs, SNP panels, mitochondrial sequence)
- The samples are **not** to be subjected to whole genome sequencing methods
- The genetic data is **not** to be uploaded and/or searched against LE and/or commercial databases (as part of the Terms and Conditions when requesting the RGTM)
  - **You agree to not attempt to re-identify the donor or donor family members**

Additions to the consent form were developed with help from the NIST Research Protections Office

https://strbase.nist.gov/NIST_Resources/RGTM_10235/ISPECIMEN_Consent_Form.pdf

Excerpts from Consent Form

Research studies may be conducted to further the advancements made in the areas of forensic genetics, human identity testing, and the validation of forensic DNA testing methods, forensic testing laboratories in the U.S. and around the world may conduct studies using your blood and blood components. A primary intent of this collection is to share DNA extracts with law enforcement laboratories for the purposes of research and the validation of forensic DNA typing methods.

Researchers may share findings with others. For example, information may be shared between forensic testing laboratories within the law enforcement community. Your identity will not be known to the researchers or others, but you should consider this possibility when considering whether you would like to participate. Genetic information collected for research purposes will not be stored in databases that are used for criminal searches and will not be used by law enforcement to investigate criminal acts.

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RGTM 10235: Forensic DNA Resource Samples

200 µL of each Sample

Sample	Format	Sex	Mixture Ratio	Carrier**	Concentration (ng/µL)***
1	Single source A	M		Y	5.1 ± 0.4
2	Single source A (degraded)*	M		Y	3.6 ± 0.3
3	Single source B	F		N	4.9 ± 0.4
4	Single source B (degraded)*	F		N	3.0 ± 0.2
5	Single source C	M		Y	5.1 ± 0.4
6	2p mixture D+E	F:M	90:10	Y	4.5 ± 0.4
7	3p mixture D+F+G	F:M:M	20:20:60	Y	4.3 ± 0.3
8	3p mixture H+G+E	F:M:M	10:30:60	Y	4.3 ± 0.4

*degraded with UV (5 min at 20 mL volumes then combined)
**Samples with carrier contain 50 ng/µL of yeast tRNA to improve nucleic acid stability
***As measured by digital PCR (dPCR): https://doi.org/10.6028/NIST.SP.260-189

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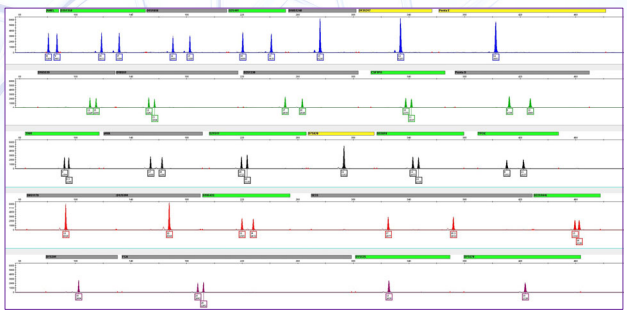
Sample 1 - PowerPlex Fusion 6C – single source



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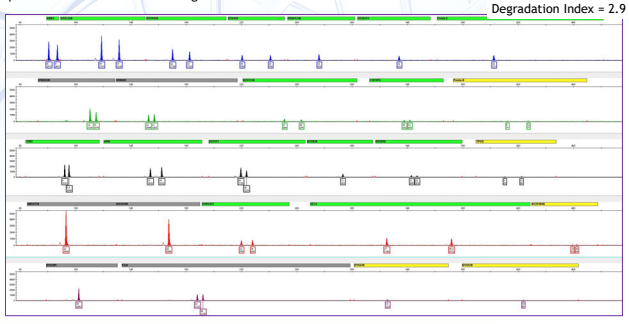
Sample 2 - PowerPlex Fusion 6C – degraded

Quantifiler Trio
Degradation Index = 2.9



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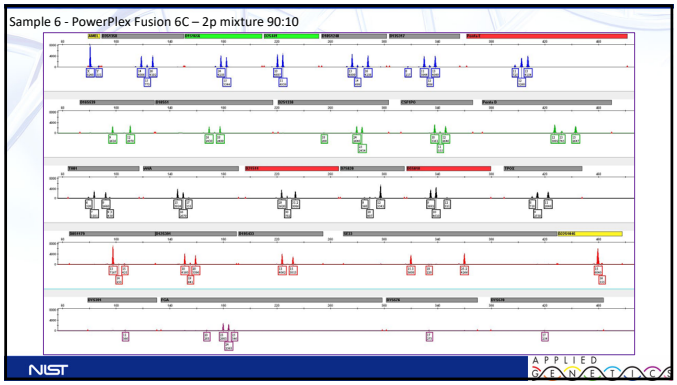
Sample 6 - PowerPlex Fusion 6C – 2p mixture 90:10
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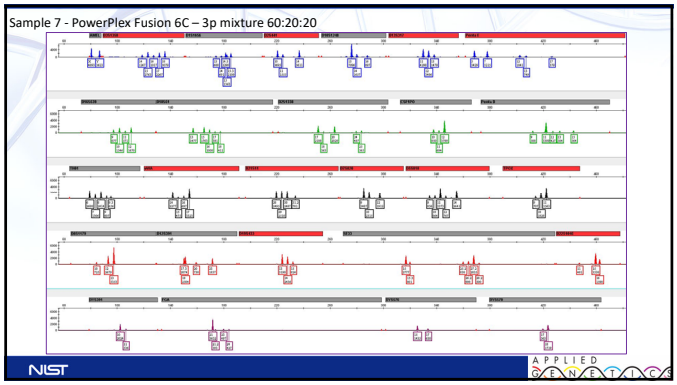
Sample 7 - PowerPlex Fusion 6C – 3p mixture 60:20:20
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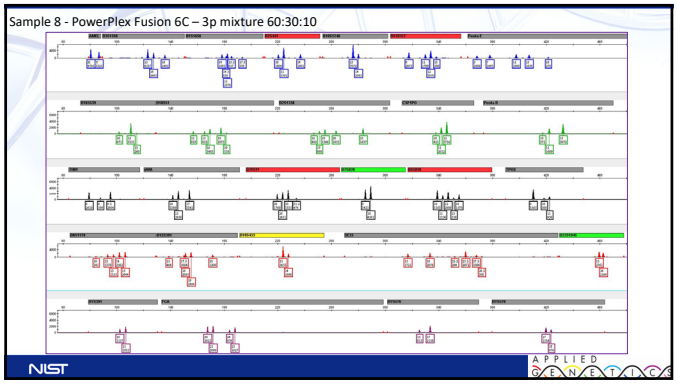
Sample 8 - PowerPlex Fusion 6C – 3p mixture 60:30:10
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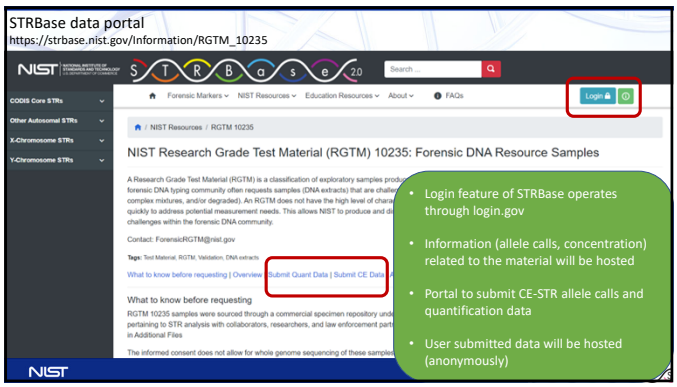
STRBase data portal
https://strbase.nist.gov/Information/RGTM_10235



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Example of Data to be reported (allele calls)

May change with future information provided

Allele calls for a degraded sample

RGTM 10235-Sample 2

	D3S1358	TH01	D21S11	D18S51	Penta_E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta_D	AMEL	vWA	D8S1179	TPOX	FGA
CONSENSUS PROFILE	16	6,7	31,2	14,16	5,16	11,12	8,12	9,11	9,11	11,12	11,14	X,Y	17,18	14	8	19,23

Sample Name	D3S1358	TH01	D21S11	D18S51	Penta_E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta_D	AMEL	vWA	D8S1179	TPOX	FGA
LAB A	16	6,7	31,2			11,12	8,12	9	9			X,Y	17,18	14	8	19
LAB B	16	6,7	31,2	14		11,12	8,12	8,11	9			X,Y	17,18	14	8	
LAB C	16	7	31,2	14	5	11,12	8,12	8,11	11	11		X,Y	17,18	14	8	

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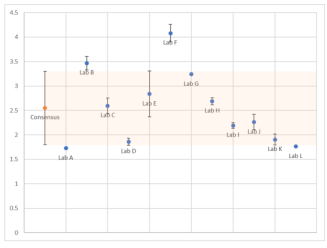
Example of Data to be reported (quantification data)

May change with future information provided

Sample 1 (ng/µL)



Quantification values from different labs/methods

Data to assess and monitor stability

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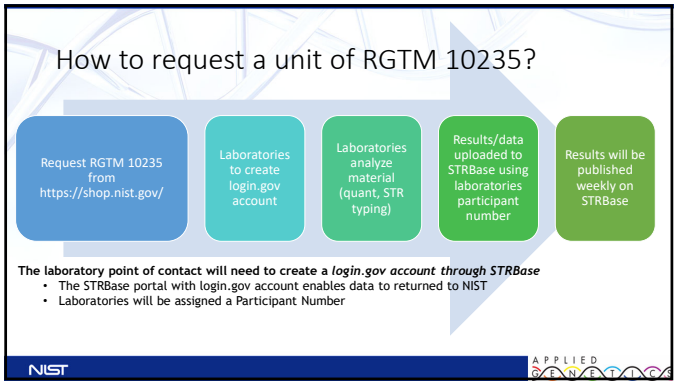
How to request a unit of RGTM 10235?
Request RGTM 10235 from https://shop.nist.gov/
Laboratories to create login.gov account
Laboratories analyze material (quant, STR typing)
Results/data uploaded to STRBase using laboratories participant number
Results will be published weekly on STRBase
The laboratory point of contact will need to create a login.gov account through STRBase
• The STRBase portal with login.gov account enables data to returned to NIST
• Laboratories will be assigned a Participant Number
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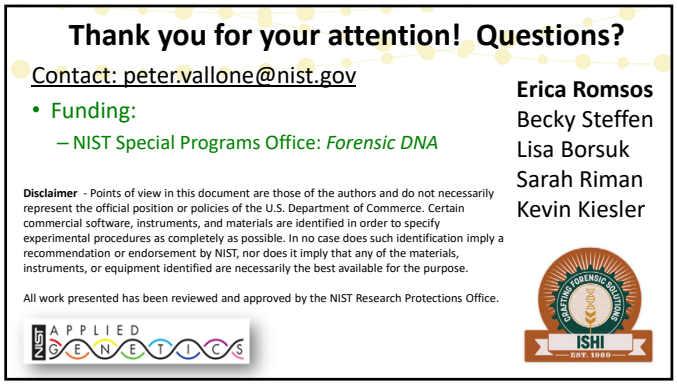
Thank you for your attention! Questions?
Contact: peter.vallone@nist.gov
• Funding:
– NIST Special Programs Office: Forensic DNA
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All work presented has been reviewed and approved by the NIST Research Protections Office.
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