

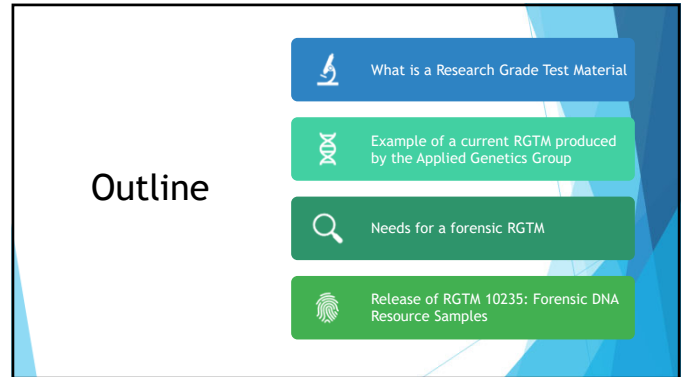


Development of a Research Grade Test Material (RGTM)
 RGTM 10235: Forensic DNA Resource Samples

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 Green Mountain DNA Conference
 July 24, 2023

APPLIED GENETICS

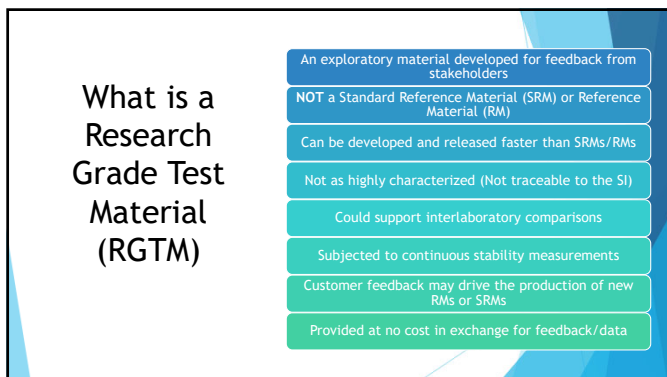
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Outline

- What is a Research Grade Test Material
- Example of a current RGTM produced by the Applied Genetics Group
- Needs for a forensic RGTM
- Release of RGTM 10235: Forensic DNA Resource Samples

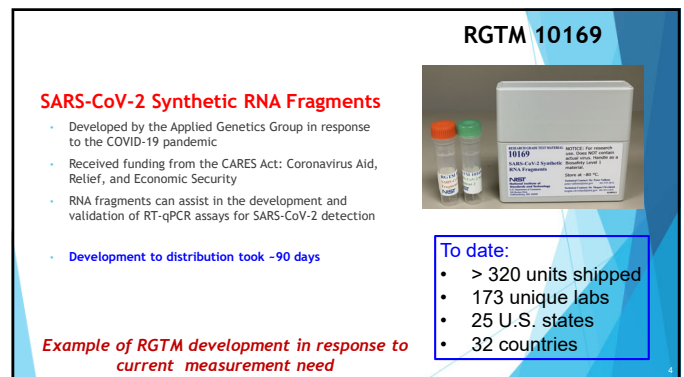
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What is a Research Grade Test Material (RGTM)

- An exploratory material developed for feedback from stakeholders
- NOT a Standard Reference Material (SRM) or Reference Material (RM)
- Can be developed and released faster than SRMs/RMs
- Not as highly characterized (Not traceable to the SI)
- Could support interlaboratory comparisons
- Subjected to continuous stability measurements
- Customer feedback may drive the production of new RMs or SRMs
- Provided at no cost in exchange for feedback/data

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RGTM 10169

SARS-CoV-2 Synthetic RNA Fragments

- Developed by the Applied Genetics Group in response to the COVID-19 pandemic
- Received funding from the CARES Act: Coronavirus Aid, Relief, and Economic Security
- RNA fragments can assist in the development and validation of RT-qPCR assays for SARS-CoV-2 detection
- Development to distribution took ~90 days

Example of RGTM development in response to current measurement need

To date:

- > 320 units shipped
- 173 unique labs
- 25 U.S. states
- 32 countries

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Why make a Forensic DNA RGTM?

- NIST receives many requests for SRMs to address ongoing measurement challenges (degradation, inhibition, complex mixtures, extraction, etc).
 - Challenges to fit within the SRM production structure needing to assess all sources and forms of uncertainty and characterization without years of stability studies
- Assist in internal validation efforts by providing a standard supplemental sample set
- Desire for more explicit informed consent
 - Samples which allow for public sharing of genotype and sequencing data and outlines intent to share with law enforcement (Crime Labs)
- Examination of new production methods to benefit future SRMs and RMs

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Benefit to NIST

- Examination of future production methods for RMs and SRMs
- NIST made changes in production methods:
 - Robotic Bottling
 - Tube type (Teflon to polypropylene)
 - Volume of DNA provided (current SRMs are 55 µL)
 - Addition of Carrier RNA and effects on stability

Each of these measurements will increase our knowledge and ability to manufacture better RMs and SRMs in the future

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Benefit to Customer

- Could assist in internal validation efforts by providing a standard supplemental sample set
- Will help inform future SRMs and RMs that are provided to the forensic DNA community
- May be used as training samples within a laboratory
- Increased informed consent allows for sharing of samples and data

RGTM 10235 is being provided at no cost to the customer in exchange for data to support long term fit for purpose measurements

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Evolution of Informed Consent

- Previous consent was generic and allowed for blood bank to "use in any manner" (i.e. sold to NIST AGG)
- Desire for a more explicit informed consent for donors
- Explicitly allows for the public sharing of genotype and sequencing data
- Outlines primary intent to share samples with law enforcement
- Allows for samples to promote research as well as become a commercial product (SRM)

Addendum was developed with help from Anne Andrews and the Research Protections Office

Addendum for the Use of Human Blood and/or Blood Components for Research

Research studies may be conducted to further the advancements made in the areas of forensic genetics, human identity testing, and the resolution of forensic DNA testing methods.

Participation in research is voluntary. By agreeing to donate blood and blood components to research any donor information provided will not individually identify you.

Research participation will not involve any cost, time, or additional procedures beyond the normal donation process.

How will my blood and blood components be used in research?

- Your blood and blood components may be used in research to lead to a better understanding and development of forensic DNA testing methods.
- Your blood and blood components may be stored indefinitely, made available to researchers, or distributed commercially.

Who may use my blood and blood components for research?

- Research staff from universities, government agencies, 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 samples with law enforcement laboratories for the purposes of research and the validation of forensic DNA testing methods.

How is my privacy protected when my blood and blood components are used in research?

- Researchers will not be given any information that would allow them to individually identify you.
- Research studies conducted with your blood and blood components are not intended for researchers to individually identify you. However, since blood contains your DNA, there is always the possibility of identification through DNA databases such as those used for genetic genealogy.

How will my blood components be used in genetic research?

- Researchers may sequence and/or examine your DNA in studies using blood and blood components. It is not expected that results could be used to individually identify you or result in discrimination by insurers or employers.
- Researchers may publish findings in scientific journals. Genetic information related to your sample may be shared as part of a presentation or scientific dissemination of results.
- Research may have other, though not direct, financial, educational, or other benefits to the research community. Your identity will not be known to the researchers or others, but you should consider the possibility when considering whether you wish 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 for law enforcement to investigate criminal acts.

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Release of RGTM 10235: Forensic DNA Resource Samples

- ▶ 8 extracted DNA samples
- ▶ Testing of different tube types
 - ▶ Teflon vs polypropylene
 - ▶ Addition of carrier RNA to low concentration samples

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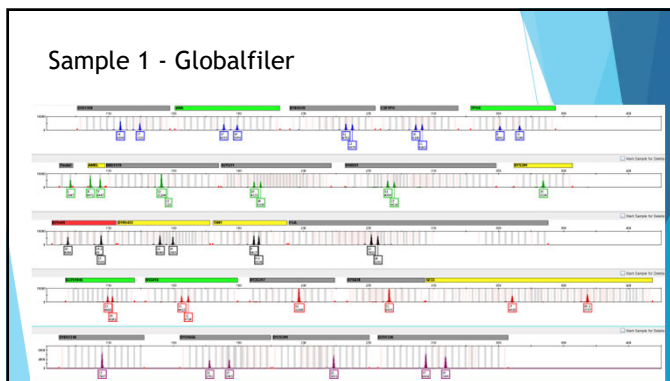
RGTM 10235: Forensic DNA Resource Samples

- ▶ Combination of single source samples and mixtures
 - ▶ Samples 1-5 are single source
 - ▶ Sample 6 is a 2-person mixture
 - ▶ Samples 7 & 8 are 3-person mixtures
- ▶ DNA in the mixtures is different than the single source samples
 - ▶ Allows for labs to create more complex mixtures with these samples for training or validation efforts

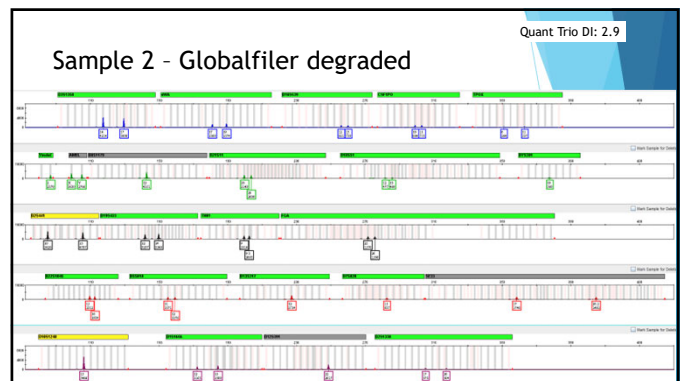
Sample	Format	Sex	Carrier**	Concentration**** (ng/μL)	Volume
Sample 1	Single source A	M	Y	5.1 ± 0.4	200 μL
Sample 2	Single source A – degraded*	M	Y	3.6 ± 0.3	200 μL
Sample 3	Single source B	F	N	4.9 ± 0.4	200 μL
Sample 4	Single source B – degraded*	F	N	3.0 ± 0.2	200 μL
Sample 5	Single source C	M	Y	5.1 ± 0.4	200 μL
Sample 6	2p mixture	M:F	Y	4.5 ± 0.4	200 μL
Sample 7	3p mixture	M:M:F	Y	4.3 ± 0.3	200 μL
Sample 8	3p mixture	M:M:F	Y	4.3 ± 0.4	200 μL

*Degraded with UV light for 5 minutes
**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>

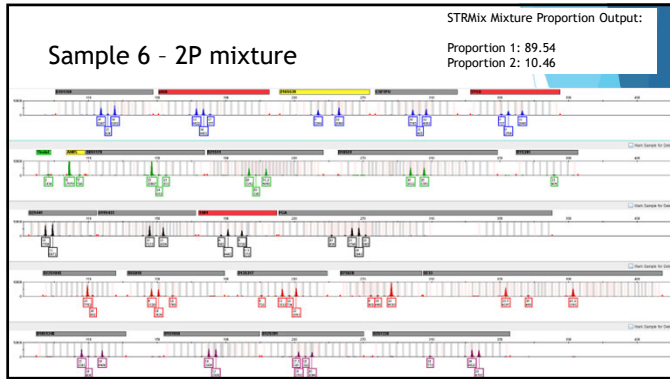
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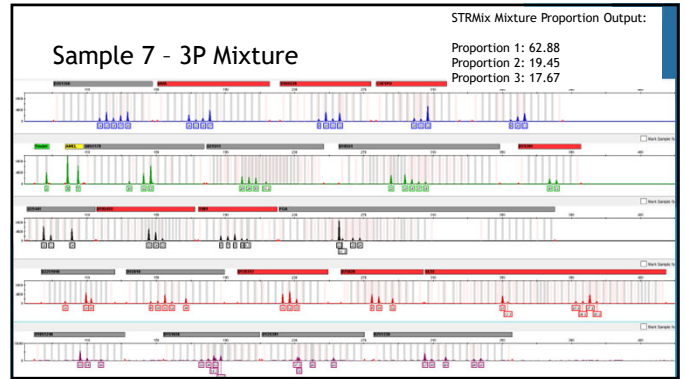
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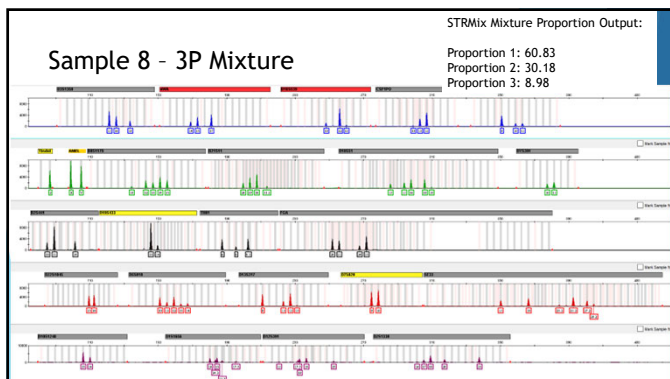
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Documentation provided to customer

Guidance documents will be provided with each unit

Further information provided on STRBase
strbase.nist.gov/Information/RGTM_10235

Forensic DNA Resource Samples
 Guidance Document
 NIST Research-Grade Test Material 10235

The research data provided is NIST Research-Grade Test Material (RGTM). This material is not a NIST Standard Reference Material or a NIST Reference Material. An outline of its use, the material and the laboratory that it was prepared in are contained in the NIST Quality Management System. RGTM RGTM is described in the latest edition of NIST Special Publication 989-204. <https://www.nist.gov/pml/xcp/989-204>

Purpose: NIST Research-Grade Test Material (RGTM) 10235 is being provided as a collaborative, research tool for purposes to evaluate potential forensic DNA programs in a reference context, by generating profiles of RGTM 10235 under laboratory conditions consistent with RGTM 10235. Material shall only be used in accordance with STRmix 4.0.0.0 (STRmix) FOR RESEARCH PURPOSES OF NIST RGTM 10235 except when reporting for internal.

Disclaimer: A set of RGTM 10235 consists of eight tubes of material from separate donor(s) and (DNA) origin, including five single source and three mixed source samples. The complete listing of samples is provided in Table 1.

Sample	Format	Sex	Carrier**	Concentration***	Volume
Sample 1	Single source A	M	Y	3.1 ± 0.4	200 µL
Sample 2	Single source A degraded†	M	Y	1.4 ± 0.1	200 µL
Sample 3	Single source B	F	N	4.9 ± 0.4	200 µL
Sample 4	Single source B degraded†	F	N	3.0 ± 0.2	200 µL
Sample 5	Single source C	M	Y	5.1 ± 0.4	200 µL
Sample 6	2p mixture	M/F	Y	4.3 ± 0.4	200 µL
Sample 7	2p mixture	M/M†	Y	4.3 ± 0.3	200 µL
Sample 8	2p mixture	M/M†	Y	4.3 ± 0.4	200 µL

*Degraded DNA (100:100) mixture.
 †Sample with average volume 10 µg/L of total DNA to improve carrier and stability.
 **As measured by digital PCR (dPCR). <https://www.nist.gov/pml/xcp/989-204>

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What kind of information can be submitted to NIST?

Customers will be required to accept Terms and Conditions at checkout and provide feedback and results to NIST

Future needs for new materials

Concentration measurements

STR genotyping results

Interpreted mixtures

- Pending fit for purpose assessment

Data portal and repository developed through STRBase to facilitate feedback and anonymous comparison between laboratories

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STRBase data portal

STRBase data portal

NIST Research Grade Test Material (RGTM) 10235: Forensic DNA Resource Samples

A Research Grade Test Material (RGTM) is a classification of exploratory samples produced by NIST which aims to evaluate fit-for-purpose needs within a community. The forensic DNA typing community often requests samples (DNA extracts) that are challenging to develop (precipitates, bedding, etc.) and characterize (low concentration, complex matrices, and/or degraded). An RGTM does not have the high level of characterization when compared to a Standard Reference Material but can be disseminated quickly to address potential measurement needs. This allows NIST to produce and distribute homogeneous and stable samples to address emerging measurement challenges within the forensic DNA community.

Contact: ForensicRGTM@nist.gov

Log: ForensicRGTM 10235: Forensic DNA Resource Samples

Login feature of STRBase operates through login.gov

This will facilitate users accounts to allow for data upload

What to know before requesting

RGTM 10235 samples were received through a commercial equipment repository under informed consent that explicitly allows for the public sharing of genetic data pertaining to STR analyses with academic, research, and law enforcement partners such as crime labs. The informed consent used for these samples may be found in Additional Files.

In requesting a set of RGTM 10235, there is an understanding that data will be provided back to NIST and will need to be returned via an upload to STRBase through the laboratory's designated subscriber. This subscriber should be a technical point of contact within the laboratory in the event submissions are incomplete or additional data is being requested. Data may be a combination of quantitative, STR genotype, and mixture deconvolution data.

RGTM 10235 will not be shipped to requesting laboratories until the verification process is complete, which will include the execution of a Material Transfer Agreement.

The data submitted will be compiled and reported outward on STRBase in an anonymized fashion through use of a participant number. Participant numbers will be assigned after the execution of a Material Transfer Agreement. An email will be sent to the Technical Point of Contact within a laboratory with the laboratory participant number and further detailed instructions.

NIST will do its best to protect the privacy of each participant and participant number. NIST may reach back to laboratories for further information about protocols or techniques to better understand the data submitted.

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STRBase account through login.gov

Submitter Information

First Name: Erica

Last Name: Romoos

Email: erica.romos@nist.gov

Organization: NIST

RGTM User ID#: RGTM User Id

- ▶ Will gain access to the RGTM 10235 upload portals
 - ▶ Provide to users after unit is requested from shop.nist.gov
- ▶ RGTM User ID# will be pre-populated through the establishment of the STRBase account

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qPCR Upload Interface

Quantification Information

Date Run: 07/23/2023

Instrument: Type and/or Select

qPCR Chemistry: Type and/or Select

Analysis Software and Version:

Upload up to 3 files:

Choose Files (No file chosen)

Notes

Additional Notes:

Guidance on desired export will be provided on STRBase

This will aid in establishing fit-for-purpose and longitudinal data

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CE upload Interface

GeneMapper export table file will be provided on STRBase to standardize upload for CE interface

Associated Quantification Information

Name of submitted quantification file:

CE Information

Date Run: 07/23/2023 Instrument: Amplification Kit:

Input DNA amount: Measurement Unit: PCR Cycle Number:

CE Injection Parameters

Injection time (second): Injection voltage (V):

Analysis Software and Version:

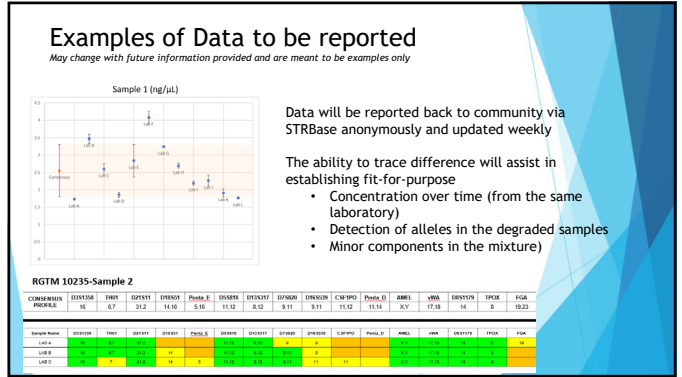
Analytical Threshold

Instructions:
Please enter an integer value for Blue, Green, Yellow, Red, Purple, and Orange

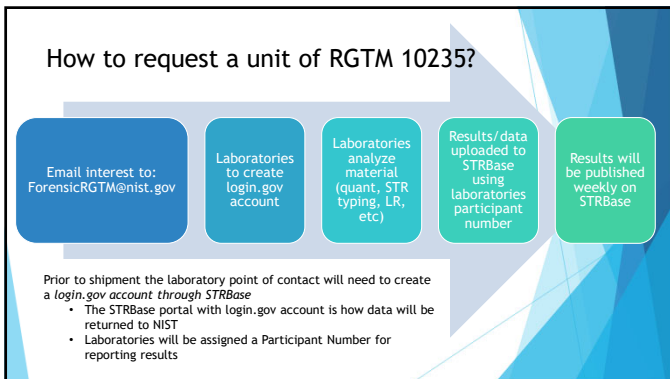
Blue: Green: Yellow:

Red: Purple: Orange:

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Thank you for your attention! Questions?

Contact: Erica.Romsos@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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