Rapid DNA Testing at NIST

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U.S. Department of Commerce

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Rapid DNA platforms

- Testing on behalf of Chris Miles DHS S&T
- ANDE (NetBio)
  - PowerPlex 16 STR chemistry

- RapidHIT 200 (IntegenX)
  - PowerPlex 16 STR and GlobalFiler Express chemistry

NIST R-DNA Interlaboratory Study **Fall 2013**

- Presented last September at BCC
- Two R-DNA developers
- Three testing sites
- A total of 350 reference buccal swabs run
- Success defined as the automated calling of the 13 core STR loci
- **Overall success = 87.4%**

Update since last year

September 2013-2014

- Run a total of 452 single source samples between both R-DNA platforms
  - 727 total (Not including negative controls, tests with non-buccal swabs)
- Success measured by concordant CODIS 13 loci called **Overall success = 84.8%**
- Two instrument upgrades for each platform
- Two software upgrades for each platform

NetBio ANDE
Instrument Status at NIST

Aug. 2013	NIST Interlaboratory Study
Sept. 2013	
Oct. 2013	Software upgrade
Nov. 2013	
Dec. 2013	
Jan. 2014	Software/Hardware upgrade New decryption software
Feb. 2014	
March 2014	
April 2014	ANDE returned to NetBio April 8, 2014 for hardware upgrade to participate in Developmental Validation study
May 2014	ANDE returned to NIST May 8, 2014 Developmental Validation Testing (30 chips, 150 samples)
June 2014	ANDE shipped to Chicago for DHS June 20, 2014

Aug. 2013 NIST Interlaboratory Study **IntegenX RapidHIT 200**

Sept. 2013 **Instrument Status at NIST**

Oct. 2013

Nov. 2013

Dec. 2013

Jan. 2014 **Hardware upgrade**

Feb. 2014

Announcement of Globalfiler Express

March 2014

April 2014

May 2014 **Instrument software and hardware upgraded to GlobalFiler Express kit**

June 2014 **Software Upgrade**
RH200 shipped to Chicago for DHS June 20, 2014

Feedback to developers

- Unlike the 2012-2013 period very little feedback was provided to the developers
  - Variation in chip production was not observed
  - Fewer full chip failures observed
  - Minor software and hardware updates
- NIST supported developers by participating in developmental validation studies
  - Accuracy and reproducibility

Participation in developmental validation studies

- IntegenX RH200 (PowerPlex 16 chemistry)
  - 100 samples (NIST provided buccal swabs)
  - Age range (~1.5 years old)
  - 10 unique individuals
  - Results contributed to concordance and aged swab study
- NetBio ANDE (PowerPlex 16 chemistry)
  - 150 samples (reference swabs) provided by NetBio
  - Samples run over 3 weeks
  - Results provided back to NetBio/GEHC electronically

DV data is in the hands of the developers in the support of peer-reviewed studies

Positive and negative control experiments to support SWGDAM

- Over the past year the FBI R-DNA committee has been developing an addendum to the QAS for databasing labs to guide the use of R-DNA
- Question: to what extent are positive and negative controls needed?
  - They occupy valuable space on the chip
  - How can positives/negatives guide decisions?
- Design and carry out experiments on positive and negative controls
  - Swab positive (buccal cells)
  - Swab negative (clean swab)

O = Known Sample
X = Blank

Control Data Experiments

- Checkerboard and Zebra Stripe patterns to assess contamination

Checkerboard Pattern

Lane	1	2	3	4	5
Chip 1	X	O	X	O	X
Chip 2	O	X	O	X	O

3 replicates of pattern

Zebra Stripe Pattern

Lane	1	2	3	4	5
Chip 1	X	X	X	X	X
Chip 2	O	O	O	O	O

2 replicates of pattern

- No contamination or sample carryover observed
- Low-level artifacts which were called were properly flagged and not transferred into CMF file

Positive and negative controls

- Presence or absence of signal from a positive or negative control is not a good indicator of the success of other lanes
- This led the recommendation that positive and negative controls are not required for every run
- However, controls will be required for
  - **Cartridge/reagents check (lot check): run a positive and negative control (before or in parallel with reference samples)** *Standard 9 Analytical Procedures*

Positive	Negative	Sample 1	Sample 2	Sample 3

  - **Performance check: run positives on all lanes** *Standard 10 Equipment Calibration and Maintenance*

Positive	Positive	Positive	Positive	Positive

Making materials traceable to NIST SRM 2391c

- SRM = standard reference material ***Reference material*** is a material for which values are certified by a technically valid procedure and is accompanied by, or traceable to, a certificate or other documentation, which is issued by a certifying body.
- QAS 9.5.5 The laboratory shall check its DNA procedures ***annually*** or ***whenever substantial changes*** are made to a procedure against an appropriate and **available NIST standard reference material** *or* ***standard traceable to a NIST standard***.

http://www.nist.gov/traceability/
From the QAS
http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs

Standard **R**eference **M**aterial 2391c : PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are cells spotted on 903 paper or FTA paper

No buccal swabs in SRM 2391c
The paper components may not contain enough cells for R-DNA analysis

Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs

Lab 1 ⟷ Lab 2

Helps meet QAS Std. 9.5.5 and ISO 17025

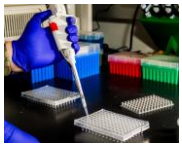
Standard Reference Material

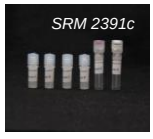
Calibration with SRMs enables confidence in comparisons of results between laboratories

How to make a NIST traceable swabs (SRM 2391c) - example

Collect a lot of 10 Buccal swabs from single individual
You are making this lot of swabs traceable to the SRM

Extract the DNA from two swabs from the lot (traditional lab methods)





Amplify extracted swabs along with components from SRM 2391c

Verify SRM 2391c allele calls are accurate against the certificate and make allele calls for the (now) traceable swab lot

How to make a NIST traceable swabs (SRM 2391c)

- These swabs can be used on R-DNA instruments now as a NIST traceable material
  - Must confirm typing results after running on a R-DNA platform
  - The process must be repeated to make another traceable lot of materials
- Use of traceable swabs:
  - **Annually or when upgrades are made (9.5.5 of QAS)** *also if desired*
  - During a critical reagents and R-DNA cartridge check (Standard 9)
  - R-DNA performance check (Standard 10)

Initial testing of GlobalFiler Express on the RapidHIT 200

PowerPlex 16

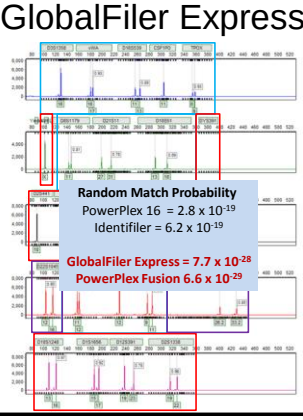
- 16 genetic markers
- Run up to 5 samples
- Stored at 4ºC
- ≈108 min runtime

GlobalFiler Express

- 24 genetic markers
- Run 1 to 7 samples
- Stored at RT (polymer at 4ºC)
- ≈2 hour runtime

GFE Released Feb 2014

GlobalFiler Express

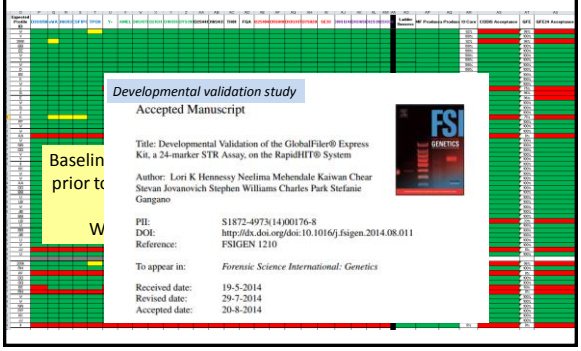


The U.S. is in the process of expanding their core loci from 13 to 20

Hares, D.R. Addendum to expanding the CODIS core loci in the United States, Forensic Science International: Genetics, Volume 6, Issue 5, September 2012, Page e135

http://www.cstl.nist.gov/strbase/pub_pres/Hill-ISFG2013-expandedUScore.pdf

GlobalFiler Express Heatmap



R-DNA Maturity Assessment

- Fall of 2014 assessment of the current status of rapid DNA typing technology for the CODIS Core Loci
- 20 reference buccal swabs will be provided to participants
- Automated or manually reviewed data submitted to NIST
- Overall success for NIST provided samples will be reported

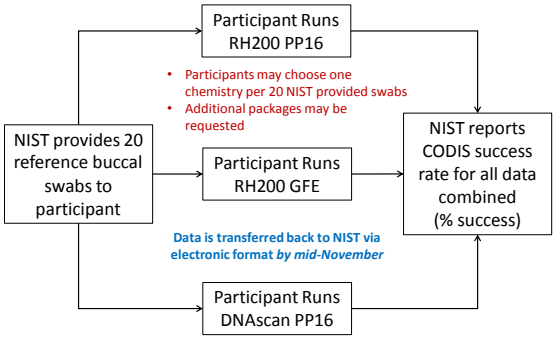
http://www.nist.gov/mml/bmd/genetics/dna_biometrics.cfm

Rapid DNA Instrument Platforms	Participating labs	Total instruments	Samples attempted	Core CODIS Success
2	5	10	200	180/200 = 90%

Example format of assessment

R-DNA Maturity Assessment

Example workflow



Thank you for your attention!

Questions?

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To request participation in the R-DNA Maturity Assessment please email erica.butts@nist.gov

Outside funding agencies:
FBI - Evaluation of Forensic DNA Typing as a Biometric Tool
DHS – Rapid DNA Prototype and Kinship Analysis Performance Evaluation