Rapid DNA Testing at NIST

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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

Instrument Status at NIST

NetBio ANDE

August 2013
- NIST Interlaboratory Study
- Software upgrade

September 2013
- Software/Hardware upgrade
- New decryption software

October 2013

November 2013

December 2013

January 2014
- ANDE returned to NetBio April 8, 2014 for hardware upgrade to participate in Developmental Validation study

February 2014

March 2014

April 2014
- ANDE returned to NIST May 8, 2014
- Developmental Validation Testing (30 chips, 150 samples)
- ANDE shipped to Chicago for DHS June 20, 2014

May 2014

June 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)

### Control Data Experiments

- **Checkerboard and Zebra Stripe patterns to assess contamination**
  - **Checkerboard Pattern**
    - Lane 1: X O O X X
    - Lane 2: O X X X X
  - **Zebra Stripe Pattern**
    - Lane 1: 1 2 3 4 5
    - Lane 2: 1 2 3 4 5

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

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

1. Collect a lot of 10 Buccal swabs from single individual
2. You are making this lot of swabs traceable to the SRM
3. Extract the DNA from two swabs from the lot (traditional lab methods)
4. Amplify extracted swabs along with components from SRM 2391c
5. Verify SRM 2391c allele calls are accurate against the certificate and make allele calls for the (now) traceable swab lot

Initial testing of GlobalFiler Express on the RapidHIT 200

- PowerPlex 16
  - 16 genetic markers
  - Run up to 5 samples
  - Stored at 4°C
  - ~108 min runtime
- GlobalFiler Express
  - 24 genetic markers
  - Run 1 to 7 samples
  - Stored at RT (polymer at 4°C)
  - ~2 hr runtime

GlobalFiler Express

- Random Match Probability
  - PowerPlex 16 = 2.8 x 10^-19
  - Identifiler = 6.2 x 10^-19
  - GlobalFiler = 7.7 x 10^-28
- PowerPlex Fusion 6.6 x 10^-29

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

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)

Standard Reference Material 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

Calibration with SRMs enables confidence in comparisons of results between laboratories

Helps meet QAS Std. 9.5.5 and ISO 17025

Current price: $636 USD

GlobalFiler Express Released Feb 2014

GlobalFiler Express Heatmap

Baseline testing of 9 chips run (63 samples) prior to RapidHit being shipped to Chicago for further DHS testing. Will continue testing GFE this fall.

Developmental validation study

Title: Developmental Validation of the GlobalFiler Express
Author: Li et al.
DOI: 10.1016/j.chroma.2014.08.011
Reference: PMID: 25215656

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/dna_biometrics.cfm

<table>
<thead>
<tr>
<th>Rapid DNA Instrument Platforms</th>
<th>Participating labs</th>
<th>Total instruments</th>
<th>Samples attempted</th>
<th>Core CODIS Success</th>
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<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td>200</td>
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Example format of assessment

R-DNA Maturity Assessment Example workflow

NIST provides 20 reference buccal swabs to participant

Participant Runs RH200 PP16

Participant Runs RH200 GFE

Participant Runs DNAscan PP16

NIST reports CODIS success rate for all data combined (% success)

Data is transferred back to NIST via electronic format by mid-November

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