FORENSICS @ NIST
November 28-30, 2012 • #NISTForensics

Rapid Forensic DNA Typing: Protocols and Instrumentation

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Forensic STR Typing

Collection

Specimen Storage

Extraction

Quantitation

Multiplex PCR

STR Typing

Interpretation of Results

Database
Storage & Searching

Calculation of Match Probability

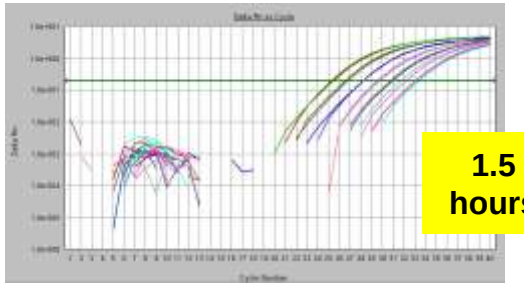
Usually 1-2 day process (a minimum of ~8 hours)

Blood Stain Buccal swab

Sample Collection & Storage

1.5 hours

DNA Extraction



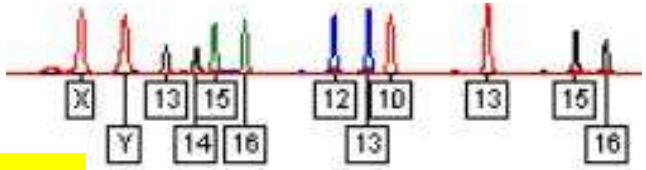
1.5 hours

DNA Quantitation

3.5 hours

Multiplex PCR Amplification

DNA separation and sizing



1.5 hours

STR Typing

Interpretation of Results

Statistics Calculated

DNA Database search
Paternity test
Reference sample

Applied Use of Information

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What is Rapid Forensic DNA Typing or Rapid DNA (R-DNA)?

- Generating a STR profile in **minutes vs hours**
  - 90 minutes versus 6-8 hours
  - Single-source reference samples (not casework)
- Non-integrated
  - Laboratory based (existing equipment)
  - Specially trained analysts
  - Robotics, fast PCR, direct PCR, quick extraction, etc
- Integrated approach
  - Fully integrated microfluidic platform
  - ‘Swab in – answer out’
  - Non-expert user

Benefits and Applications

- Faster sample-to-answer turnaround times
- Increased throughput for databasing labs

- Impact of Integrated R-DNA platforms
  - **Booking stations**, investigative leads
  - Rapid intelligence, field testing
  - Mass fatality, disaster victim investigation
  - Kinship determination, immigration, border security
  - Interest in R-DNA by FBI, DHS, DoD

Important Questions

- Can a quality result be obtained with rapid techniques?
  - Uphold DNA as the gold standard for human identification
  - Reference/database or casework samples?
  - How do we validate rapid techniques and instruments?

- Robustness
- Reliability
- Reproducibility

- Concordance ‘the correct answer’
- Sensitivity
- Contamination, mixtures
- Stutter, peak height balance, artifacts

Ongoing Projects that Support R-DNA

- Non-integrated
  - Developing rapid PCR protocols for STR kits
  - Faster thermal cyclers and DNA polymerases
  - Direct PCR kit evaluation
  - **Rapid typing workflows (Sampling through Profile)**
- Integrated approach
  - Performance assessment of prototype R-DNA instruments
  - Inter-laboratory study

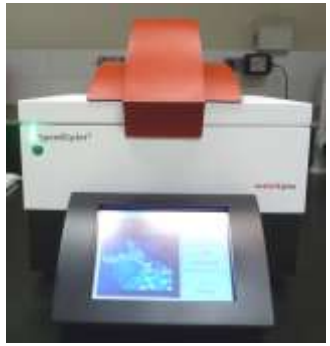
Rapid PCR Protocols

- Reducing the time required for PCR
  - 3 hours down to sub-30 minute
- Accomplish this by optimizing conditions for:
  - Faster DNA polymerases
  - Faster thermal cyclers









PCR Thermal Cyclers

Cycler	Cycling Time (min)
GeneAmp 9700	36
Mastercycler Pro S	19
Rotor-Gene Q	36
SmartCycler	22
Philisa	17
Piko	30
SpeedCycler2	22
Palm PCR	17

95°C for 1 min

28x { 95°C for 5s
58°C for 10s
72°C for 10s }

72°C 1 min

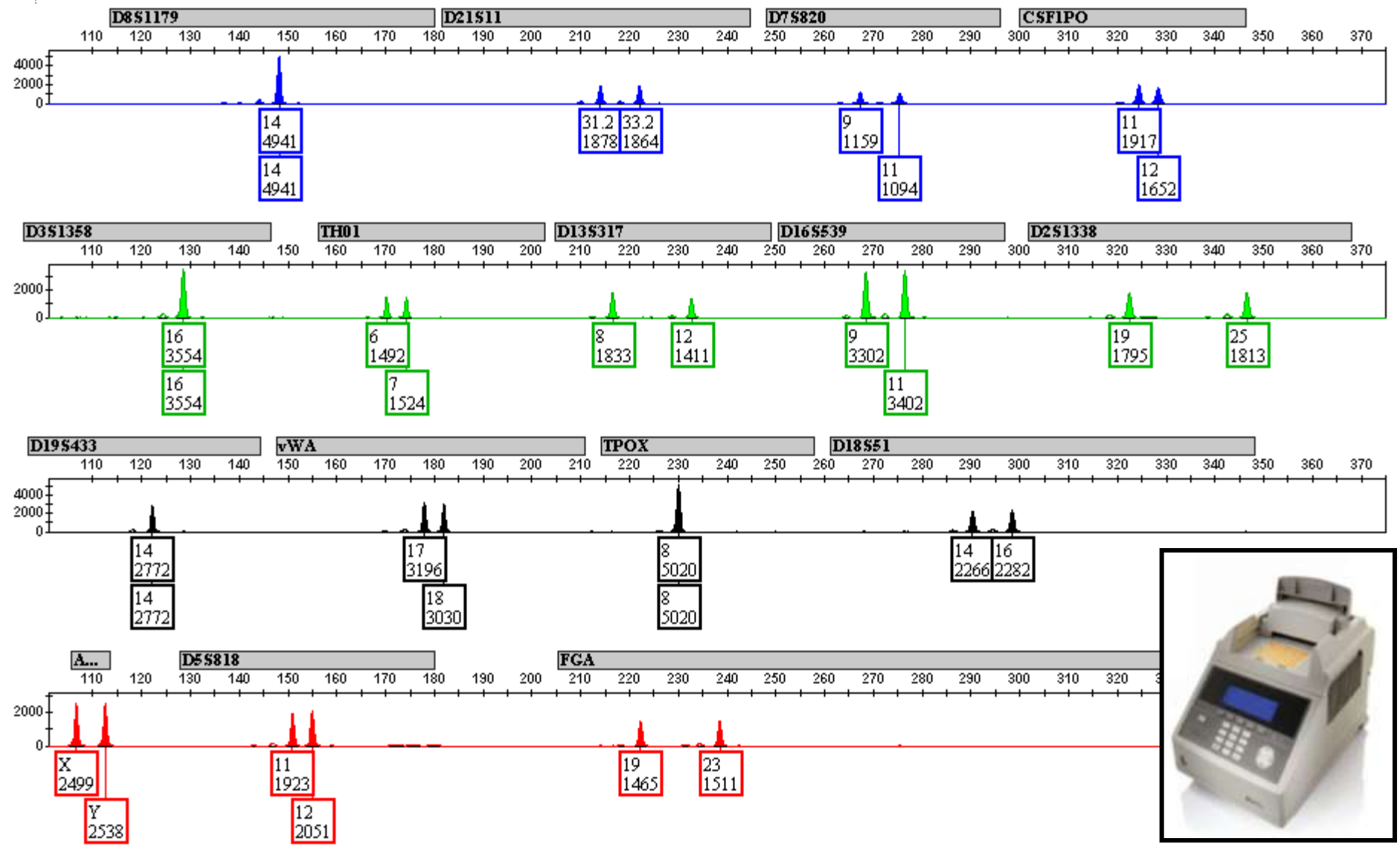
Peter Vallone: Green Mountain DNA Conference (Burlington, VT), August 3, 2012, "Development of Protocols for Rapid Amplification of STR Typing Kits: The Use of 'Non-Standard' Thermal Cyclers"

DNA Polymerases

- AmpliTaq Gold® is typically used
  - Heat activated (avoid non-specific PCR products)
- SpeedSTAR™ HS DNA Polymerase
  - Extension times of 100 bp/s are possible (compared to 20 bp/s for other polymerases)
  - Hot-start formulation is antibody mediated
- Qiagen
  - QIAGEN Fast Cycling PCR Kit
- New England Biolabs/Finnzymes
  - Phusion and Phire DNA Polymerases
- KAPA Biosystems
  - KAPA2G Fast PCR Kits
- Biotium
  - Cheetah™ Taq
- Fermentas
  - ~~PyroStart Master Mix~~
- EMD Millipore
  - KOD DNA Polymerse

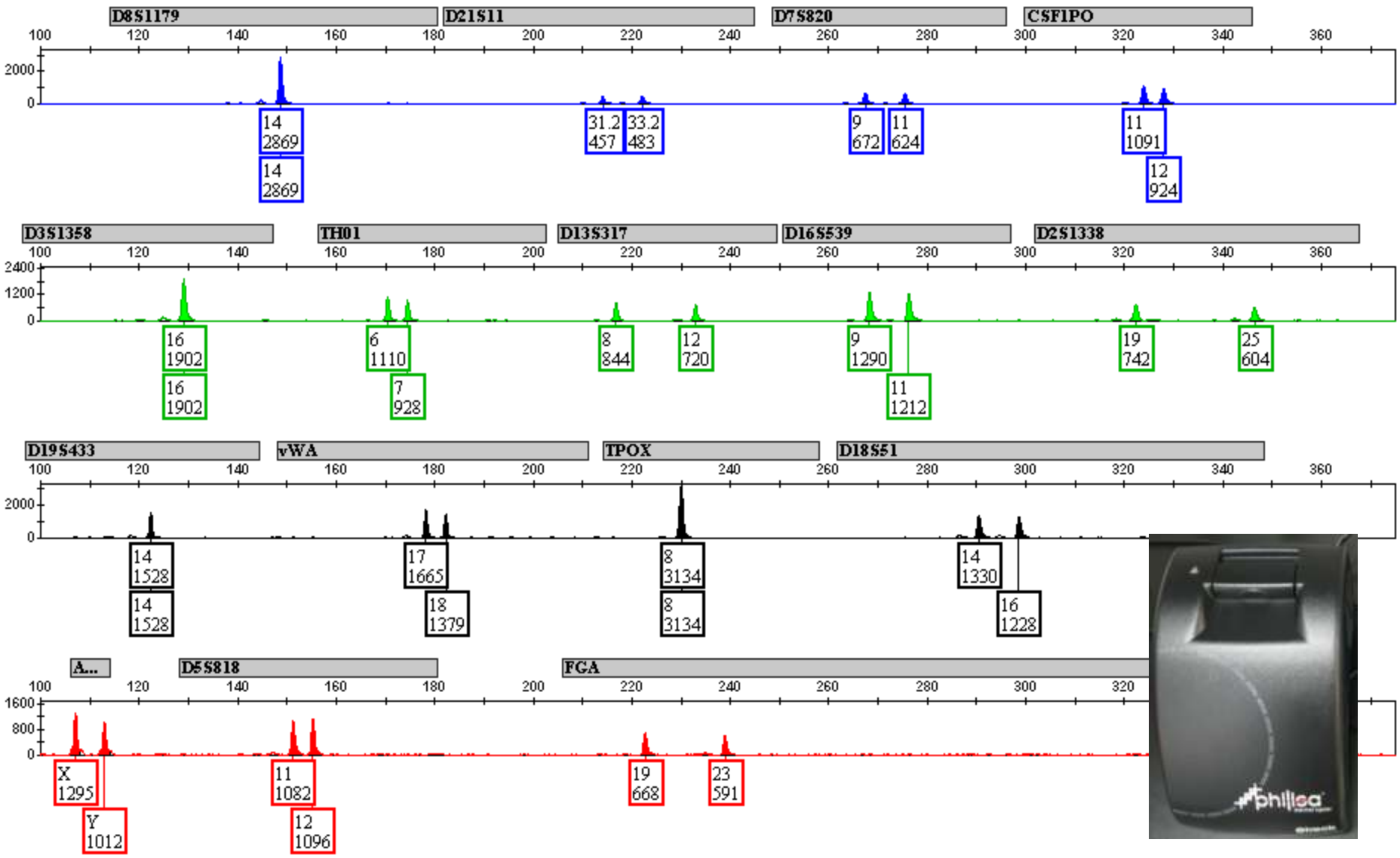
1 ng of DNA template

GeneAmp 9700 31 min PCR



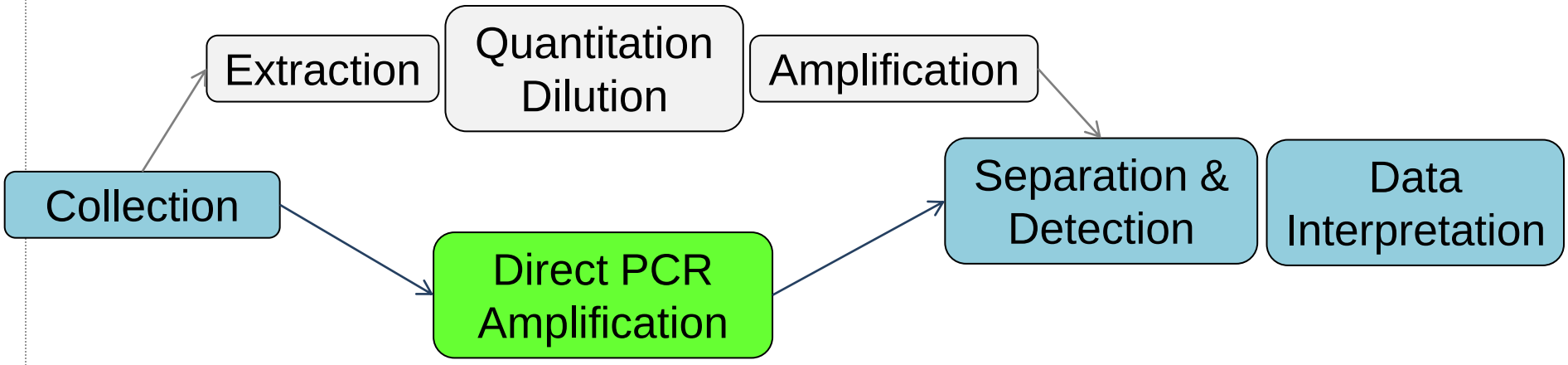
1 ng of DNA template

Philisa 17 min PCR

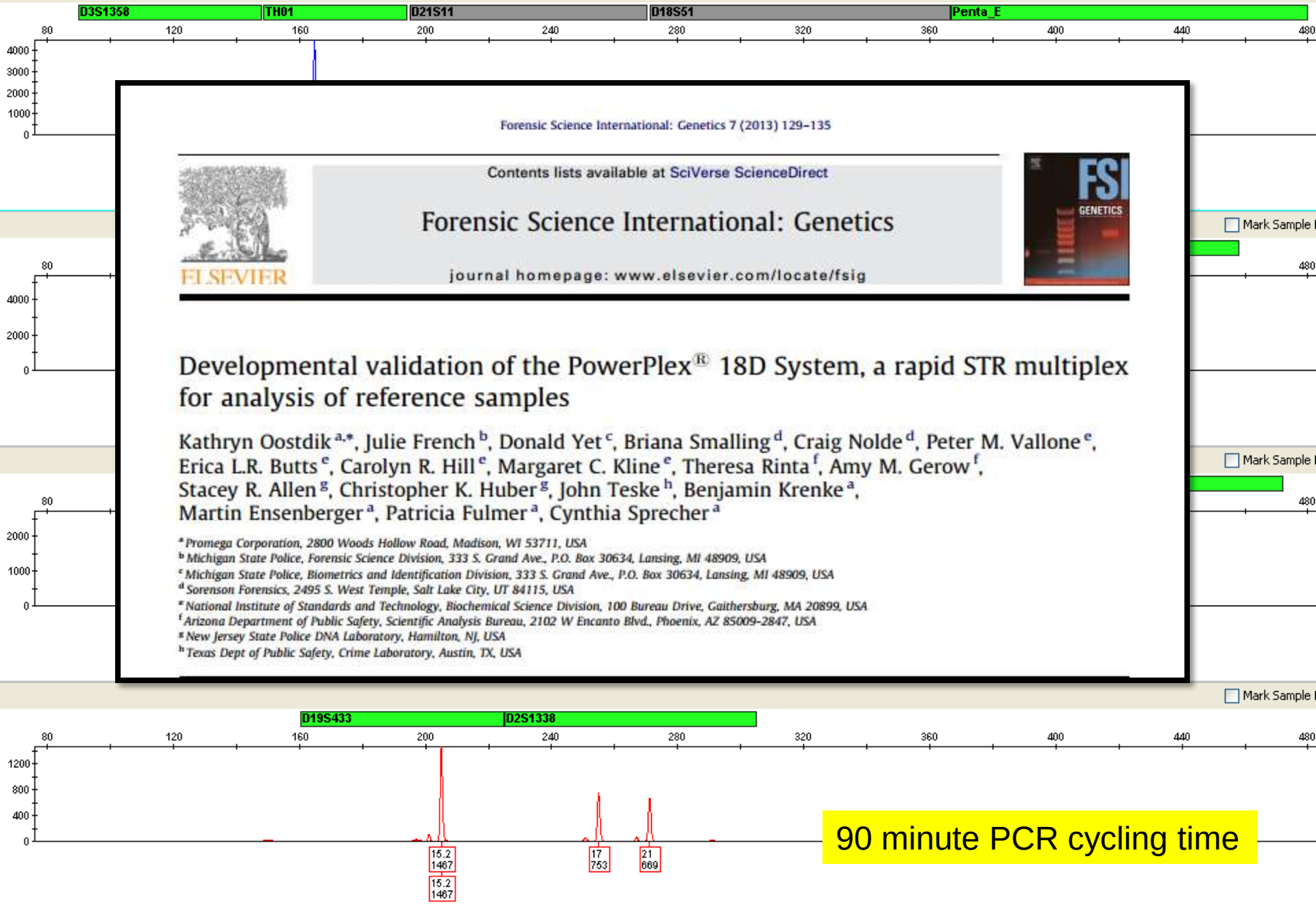


Benefits of Direct PCR

- Sample set-up convenience: ‘punch and go’
- Amplify unpurified DNA - skip extraction and quantitation
- Amenable to automation
- Applications: offender DNA database samples, paternity samples, casework reference samples



PowerPlex 18D: 1.2 mm Blood punch off FTA paper



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Developmental validation of the PowerPlex® 18D System, a rapid STR multiplex for analysis of reference samples

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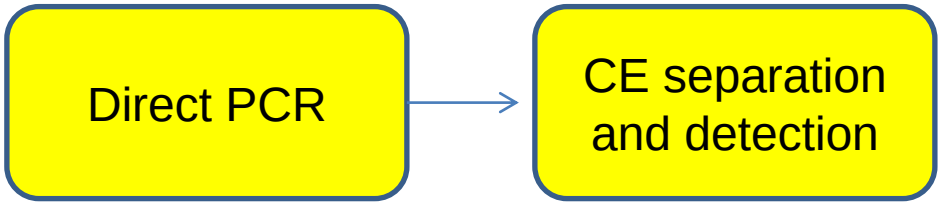
90 minute PCR cycling time

Example Rapid Typing Workflow
Non-Integrated (Lab) Setting

single source reference samples

- Extraction: Prep-N-Go Buffer
- PCR: Rapid Identifiler (Philisa cycler)
- Separations: 8 capillary 3500 Genetic Analyzer



- PP18D (9700 cycler)
- Separations: 8 capillary 3500 Genetic Analyzer

8 unique samples were typed in parallel

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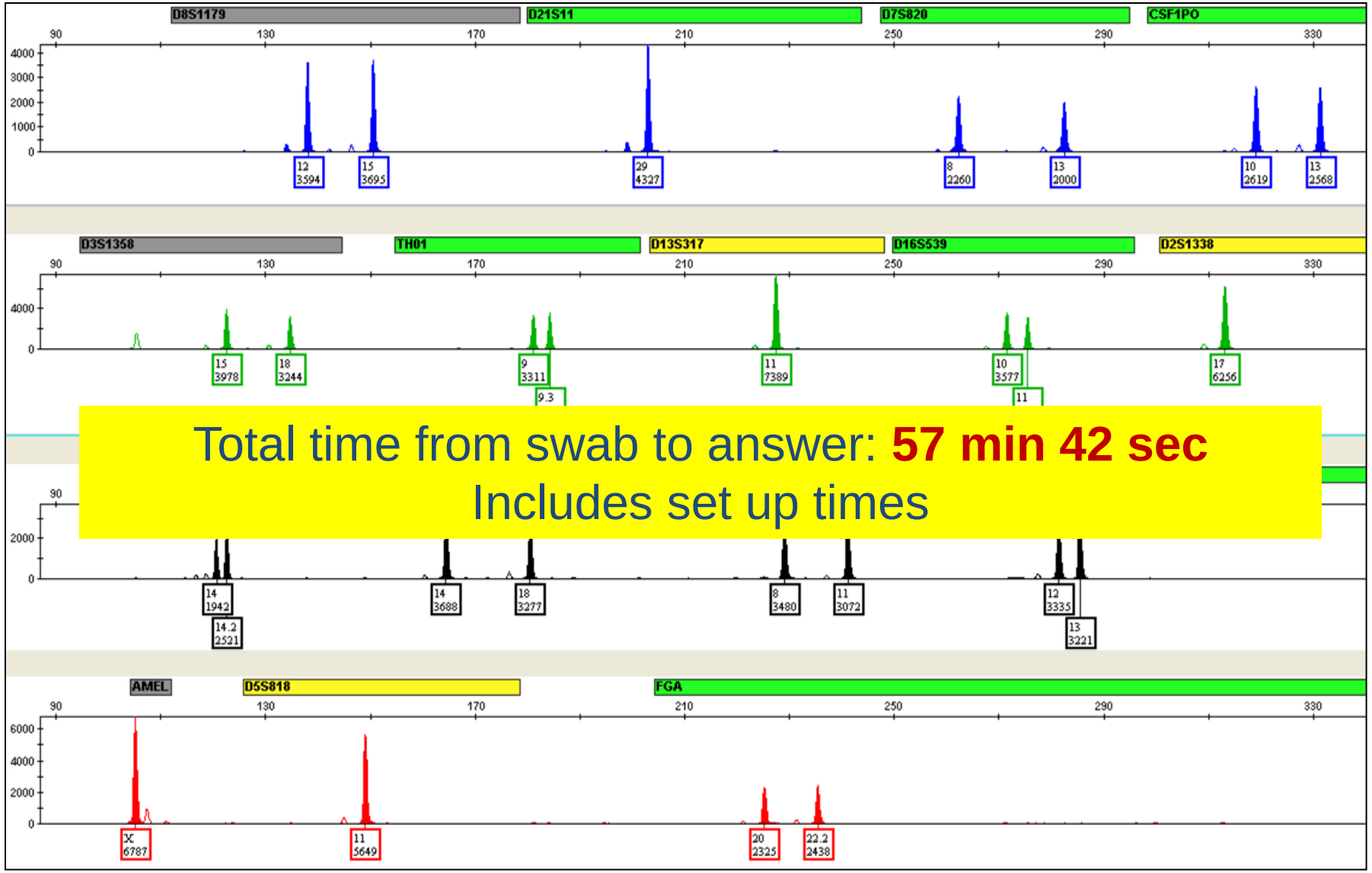
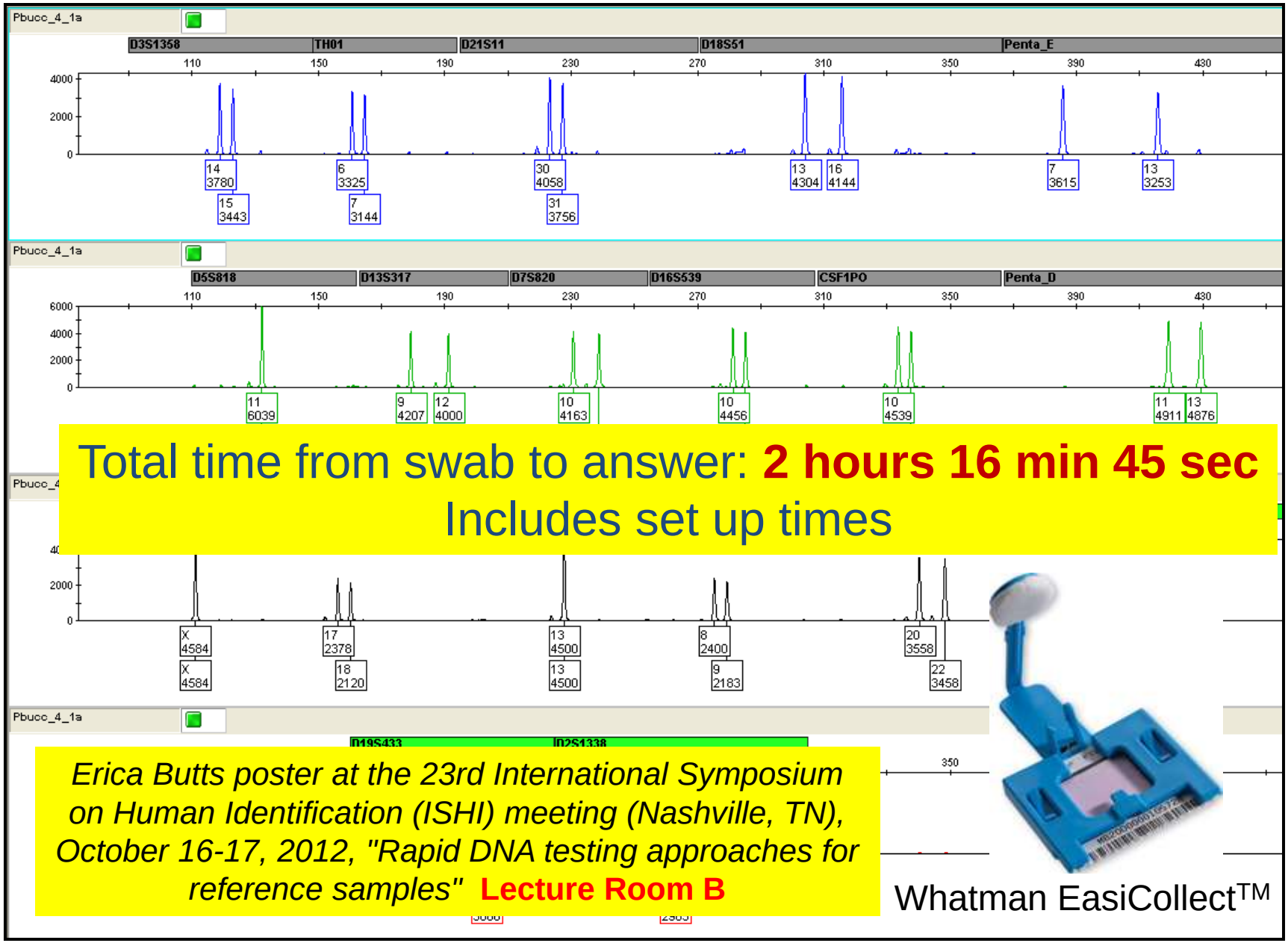
Extraction → rPCR → Separation/Detection

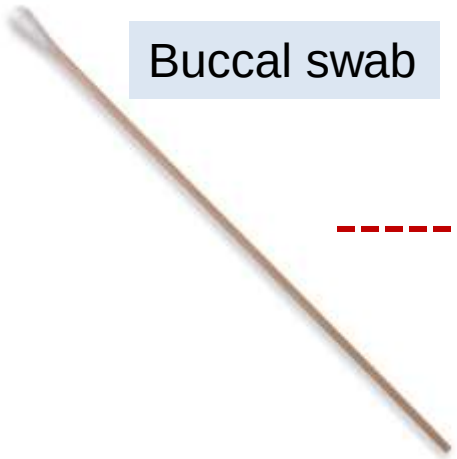


Direct PCR → Separation/Detection



Integrated Approach to Rapid DNA

Fully automated (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab



Buccal swab

R-DNA Instrument

DNA extraction
PCR
Separation and Detection
Allele calling

D8S1179	{15,16}
D21S11	{29,29}
D7S820	{9,11}
CSF1PO	{10,11}
D3S1358	{16,17}
TH01	{6,7}
D13S317	{8,12}
D16S539	{10,11}
VWA	{15,17}
TPOX	{8,12}
D18S51	{11,15}
D5S818	{9,11}
FGA	{19,22}
Amel	{X,Y}

Developers of R-DNA Instrumentation

- IntegenX
- NetBio
- ZyGem/Lockheed Martin
- Univ of Az

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Performance Testing Goals

- Testing of R-DNA platforms for baseline performance of **Robustness**, **Reliability, and Reproducibility**

- Type similar sample sets on multiple instruments and from multiple vendors
- Results will help guide platform improvements and additional testing

Carry this out through an
inter-laboratory study

NIST Inter-laboratory Test Samples

- **50 samples (buccal swabs) will be provided to each participant**
  - Five replicates of 10 anonymous individuals
  - **NIST IRB approval**
  - Each individual typed at NIST (PowerPlex 16 HS)

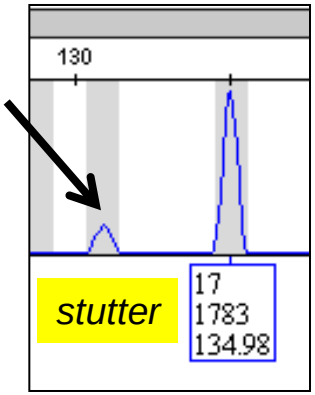
What will this data provide?
High level

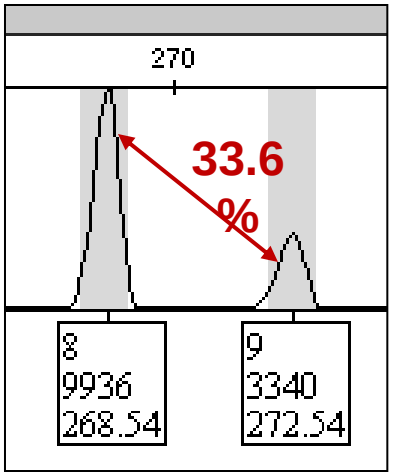
- Is the correct profile obtained?
- Typing success
  - Per lane, chip, overall
- Incorrect profiles
- Partial profiles
- Allele drop out
- Contamination
- General operational issues
  - Instrument/chip failures
  - Hardware and software

Total Runs	**44**
Total Lanes	**220**
Lanes with correct CODIS 13	90
% CODIS 13 loci	41%
Lanes with correct PP16	82
% PP16 loci	37%
Failed lanes (CODIS 13)	130
Failed chip eq	26

What will this data provide?
Detailed-expert user; developer

- Electropherogram characteristics
  - Signal intensity
  - Peak balance (inter- and intra locus)
  - Stutter, PCR artifacts, adenylation
  - Sizing precision of peaks
- Manual versus automated allele calls
  - Confirm optimal software allele calling parameters



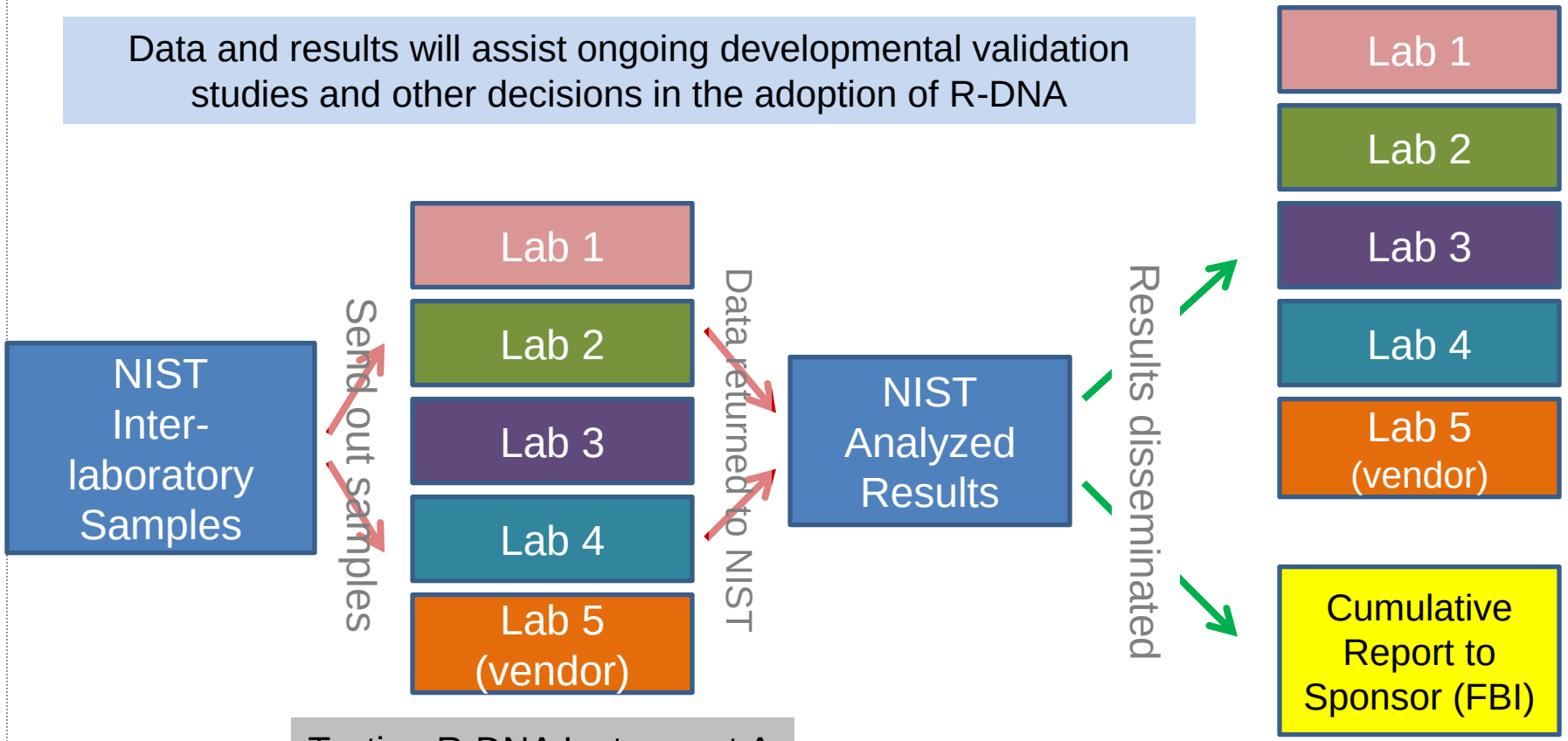


Peak balance

Inter-laboratory Testing Results

- Provide participants and sponsor with data and feedback
  - ✓ Each **participant** and will receive their specific performance feedback
  - ✓ The **sponsor** (FBI) will get a cumulative report for dissemination

Data and results will assist ongoing developmental validation studies and other decisions in the adoption of R-DNA



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Erica Butts

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Initial rapid PCR work

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