

ISHI

INTERNATIONAL SYMPOSIUM
ON HUMAN IDENTIFICATION
PHOENIX, AZ • SEP. 29–OCT. 2, 2014



**New Autosomal and Y-STR Loci and
Kits: Making Data Driven Decisions**

Internal Validation of PowerPlex[®] Fusion

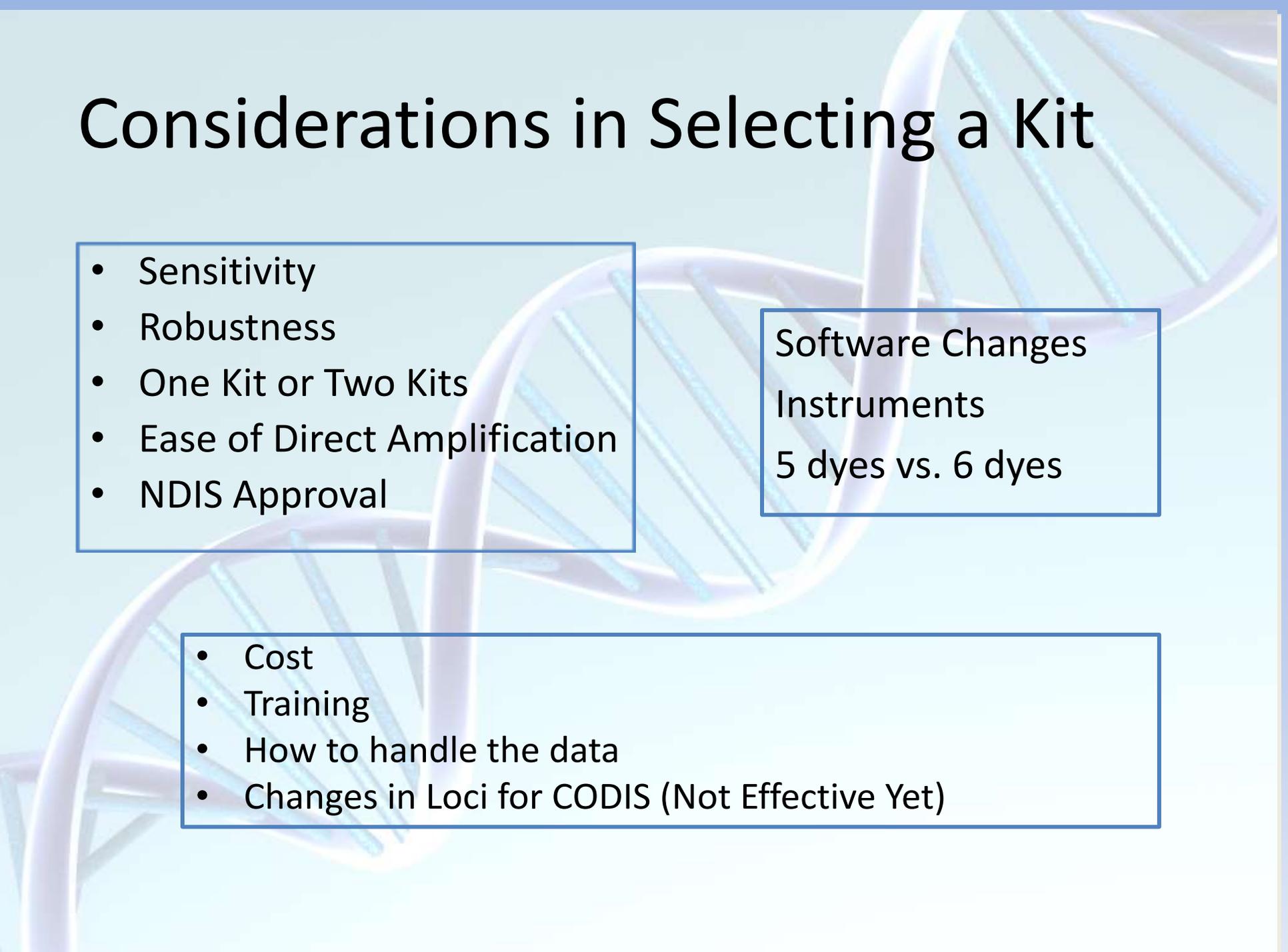


**Hope Olson
ND Office of Attorney General
Crime Laboratory Division**

History of Kits in ND

- 2000 AmpFℓISTR® Cofiler/Profiler Casework and Database, 310 Genetic Analyzer
- 2007 AmpFℓISTR® Identifiler Database, 3130 Genetic Analyzer
- 2008 AmpFℓISTR® Identifiler Casework, 3130 Genetic Analyzer
- 2008 Y-Filer™ Casework, 3130 Genetic Analyzer
- 2013 Direct Amp Fusion Database, 3500 Genetic Analyzer
- 2014 PowerPlex® Fusion Casework, 3500 Genetic Analyzer, and ArmedXpert™

Considerations in Selecting a Kit



- Sensitivity
- Robustness
- One Kit or Two Kits
- Ease of Direct Amplification
- NDIS Approval

Software Changes
Instruments
5 dyes vs. 6 dyes

- Cost
- Training
- How to handle the data
- Changes in Loci for CODIS (Not Effective Yet)

Change?

- ND's IT policy mandates that every computer had to be compatible with Windows 7 - April, 2014
- Platforms had to change while still processing casework (3130 Genetic Analyzers and GMID to 3500 Genetic Analyzer with GMID-X)
- Purchased one 3500 Genetic Analyzer and started training two new analysts last year
- We just purchased a second 3500 Genetic Analyzer in June, 2014

Internal Validation

- Database and Casework

Studies completed:

- Optimized Cycle Number
- Precision and Reproducibility
- Known and Mock Case Samples
- Sensitivity and Stochastic
- Mixture Studies
- Contamination Assessment

Optimized Cycle Number Database

- Direct Amplification Procedure
 - Add 400 μl Swab Solution
 - Incubate for 60 minutes at 90°C
 - Amplify 1 μl extract
 - Half Reaction Volume
 - Amplify using 25 cycles
 - We tried 25, 26, and 27 cycles

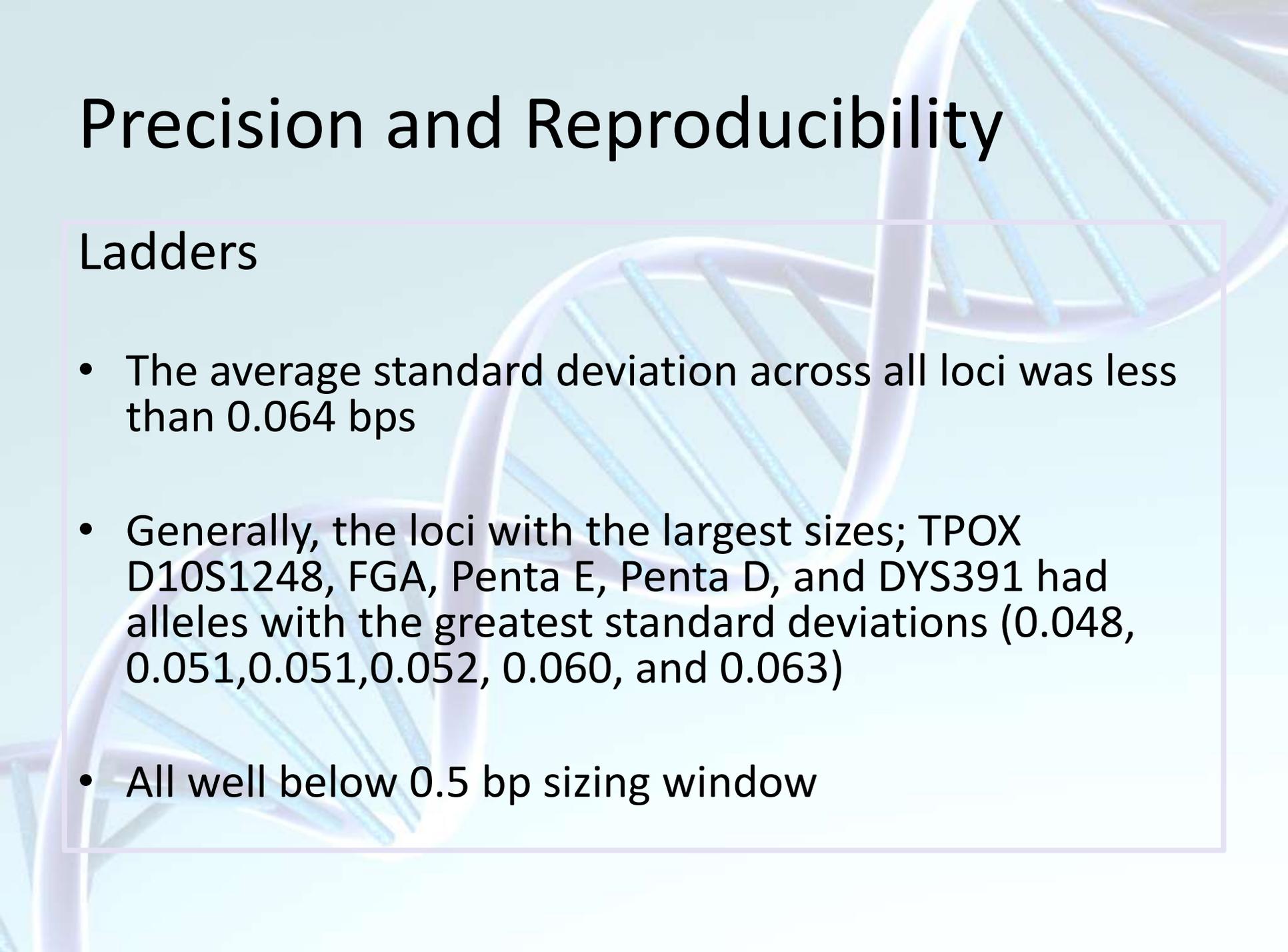
Optimized Cycle Number Casework

- Started out validating 30 cycles
- Evaluated data and reduced cycle number to 29
- Full Reaction Volume

Precision and Reproducibility

- Ladders were injected 74 times – all capillaries were tested
- A report was generated from the Report Manager in GMID®-X and imported as a tab delimited file for import into Excel
- Average bp size and standard deviation were calculated for each allele for each locus
- The average standard deviation across all loci was calculated as well

Precision and Reproducibility



Ladders

- The average standard deviation across all loci was less than 0.064 bps
- Generally, the loci with the largest sizes; TPOX, D10S1248, FGA, Penta E, Penta D, and DYS391 had alleles with the greatest standard deviations (0.048, 0.051, 0.051, 0.052, 0.060, and 0.063)
- All well below 0.5 bp sizing window

Precision and Reproducibility

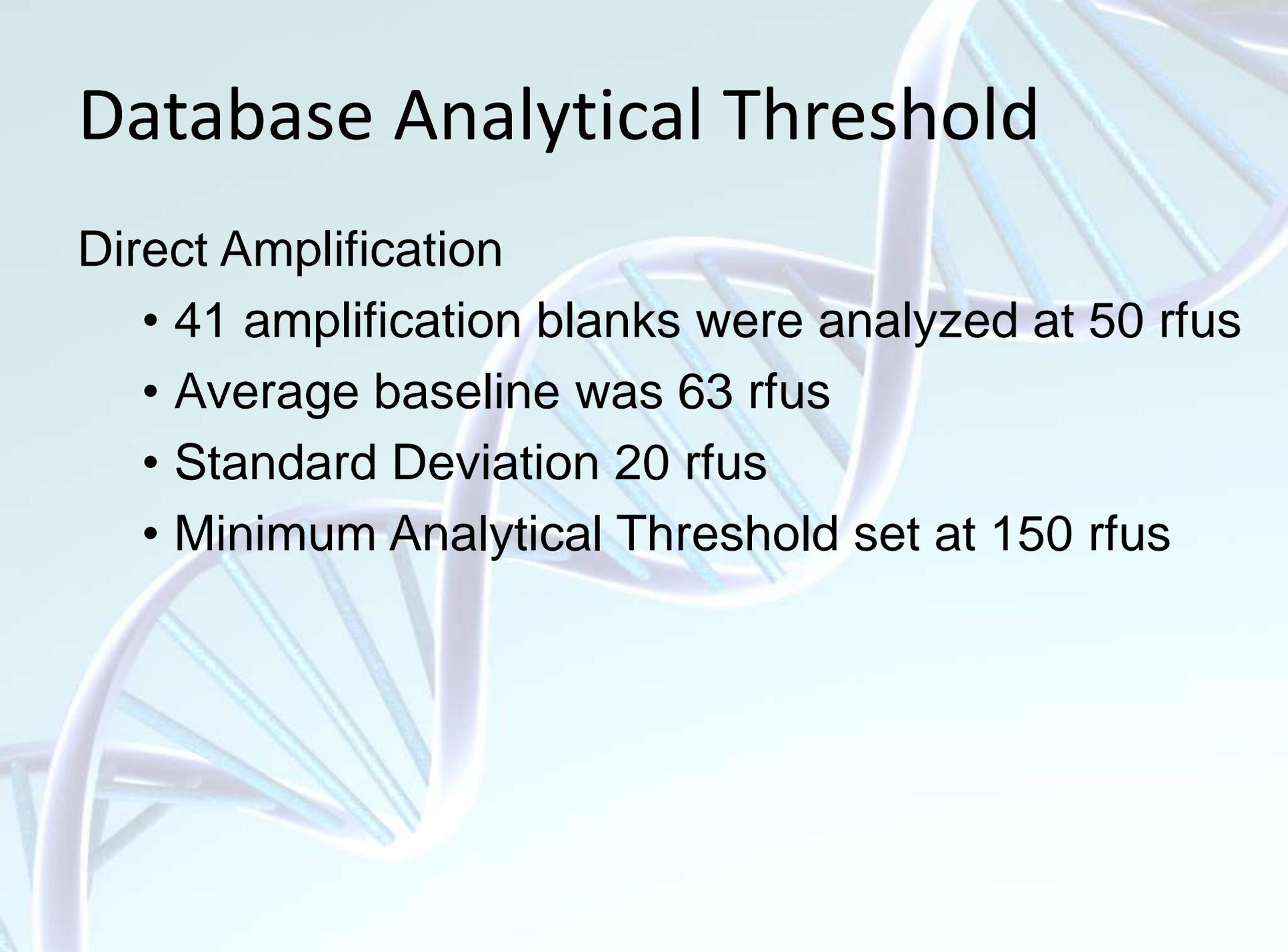
Database Known Samples

- NIST Standard 2391 B was injected at 8, 12, 18, and 24 seconds
 - Average std deviation across all loci was 0.027
- Positive Control 2800 injected 8 times over different time periods
 - Average std deviation across all loci was 0.105 bps
 - Range of values 0.036 to 0.156 bps
 - Values taken from two different columns
- Both Sample Sets demonstrated precision and reproducibility
- 46 Previously Analyzed Known Samples were compared with AmpF Φ STR® Identifiler were concordant at the loci examined

Precision and Reproducibility Casework Known Samples

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- 36 Previously Analyzed Known Samples were compared with AmpFℓISTR[®] Identifiler were concordant at the loci examined

Database Analytical Threshold



Direct Amplification

- 41 amplification blanks were analyzed at 50 rfus
- Average baseline was 63 rfus
- Standard Deviation 20 rfus
- Minimum Analytical Threshold set at 150 rfus

Casework Analytical Threshold

17 amplification blanks were analyzed at 25 rfus

Each Dye Channel was evaluated:

- Blue (37 rfus average; 12 rfus std dev) = 73 (3 std dev + mean)
- Green (42 rfus average; 23 rfus std dev) = 111 (3 std dev + mean)
- Red (41 rfus average; 27 rfus std dev) = 122 (3 std dev + mean)
- Yellow (37 rfus average; 16 rfus std dev) = 85 (3 std dev + mean)

The analytical threshold for analyzing casework was set to 150 rfus to encompass three standard deviations plus the mean for each of the dye channels

Casework Analytical Threshold

Additional samples were reviewed to determine if 150rfus was reasonable

- IQ Extraction Blanks
- Differential Extraction Blanks
- Amplification Blanks

Over 100 samples were reviewed and the average +3 standard deviations were lower than 150rfus

-After watching the NIST validation seminar the AT will be re-evaluated using a different sample set and determine if it should be adjusted

Database Stochastic

Direct Amplification

- NIST Standard 2391 A, B, and C were amplified in triplicate using 7.8, 15.6, 31.25, 63.5, 125, 250, 500, and 1000pg
- Samples run at 8, 12, 18, and 24 second injections
- Report Manger in GeneMapper[®]ID-X was used and a tab delimited file was imported into Excel to determine peak height ratios and drop-out for the sister allele

Database Stochastic

Direct Amplification

- NIST Standard 2391 Components A, B, and C had maximum stochastic values of 305, 320, and 420
- Peak height ratios were set at 60%
- An additional study was performed by varying the amount of the buccal swab in duplicate which yielded a stochastic level of 484
 - Stochastic level was set at 550 rfus

Casework Stochastic

- Three known samples were amplified in triplicate using 31.25, 65.5, 125, 250, 500, and 1000 picograms
- Run on a 3500 Genetic Analyzer utilizing four different injection parameters (8, 12, 18, and 24 seconds)

Casework Stochastic

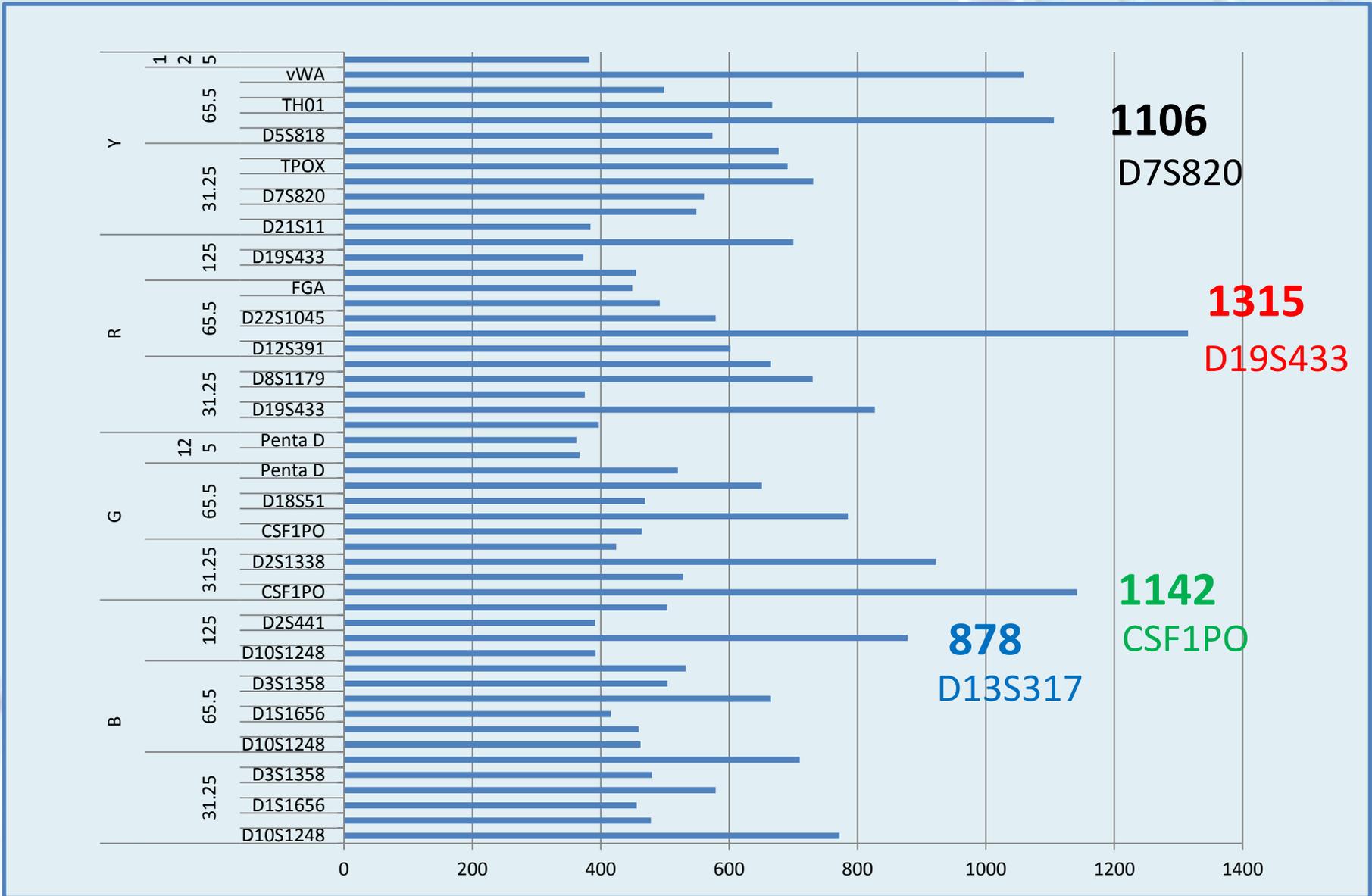
Reviewing each individual dye channel the following levels were identified:

- Blue 878 rfus
- Green 1142 rfus
- Yellow 1106 rfus
- Red 1315 rfus

Level was set at 1350 rfus, PHR set at 63%

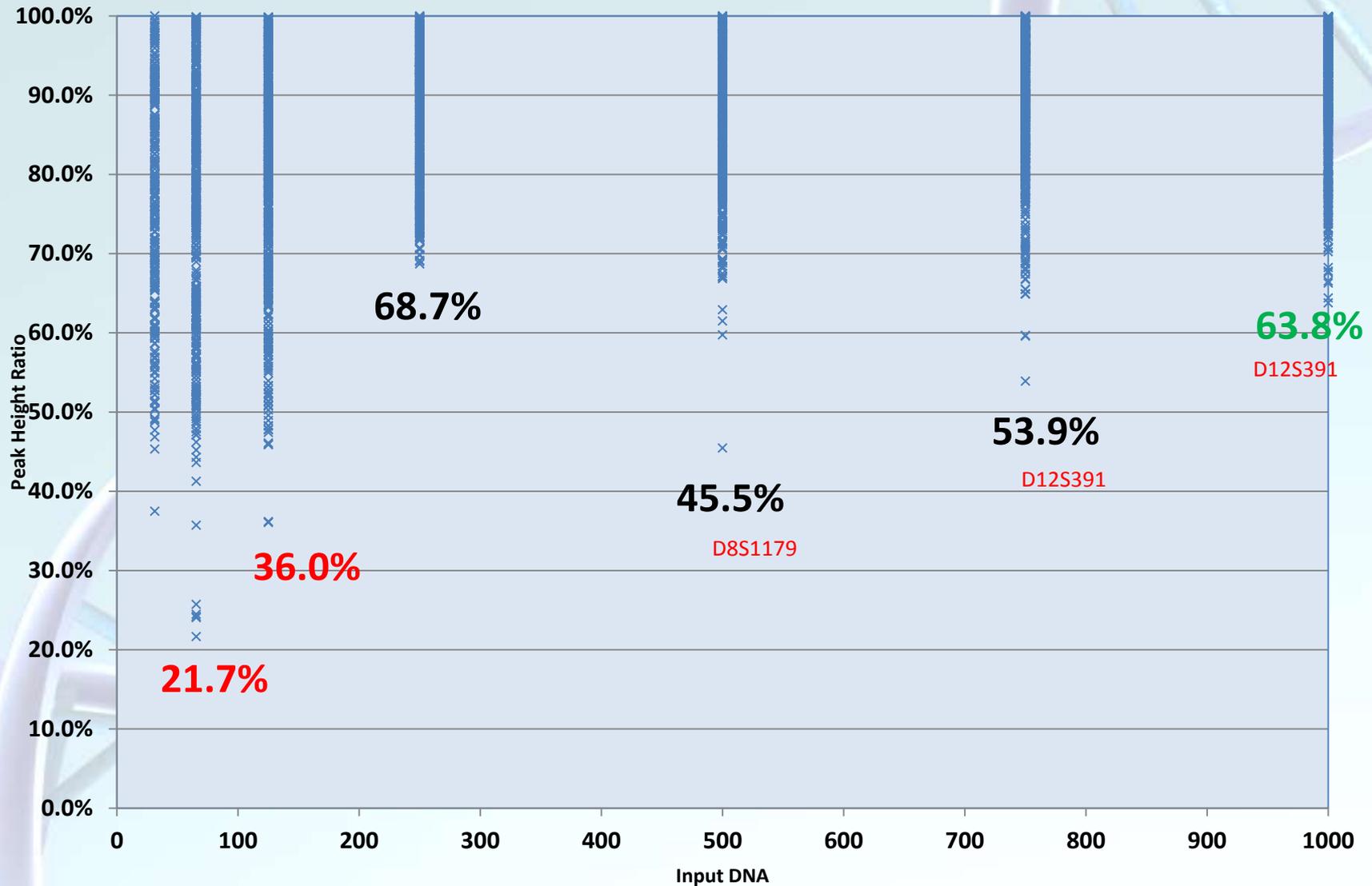
Target Amount 250 pg to 1000 pg

Input DNA vs. Drop-out of the Sister Allele



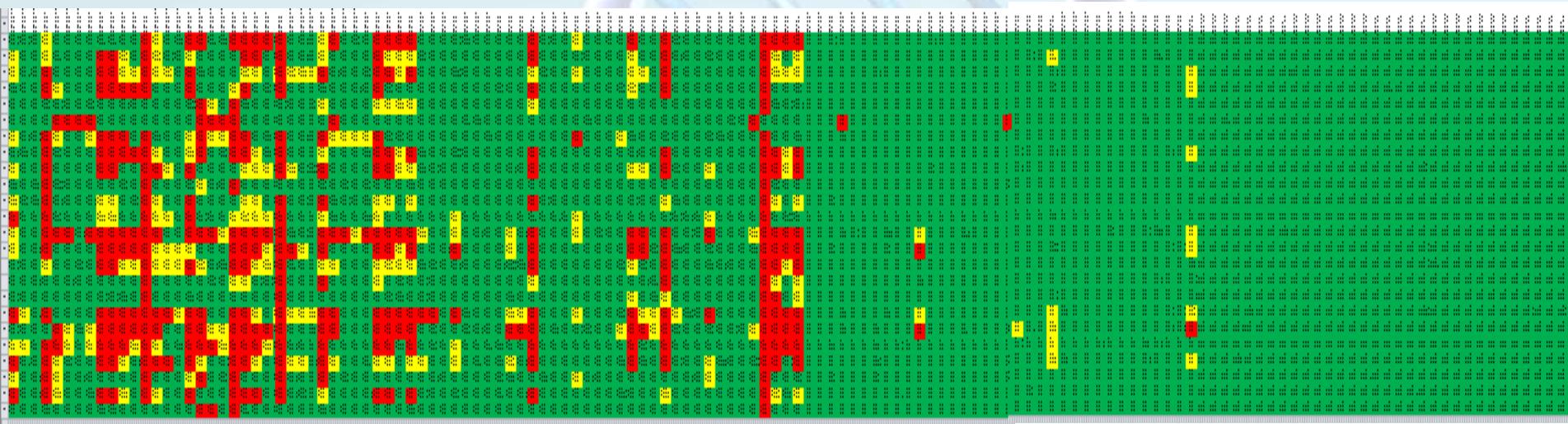
Casework Stochastic

Peak Height Ratio vs Input DNA



Casework Stochastic

Looking at the heat maps full profiles were consistently obtained at 125 picograms (Some even at **65.5 picograms**)



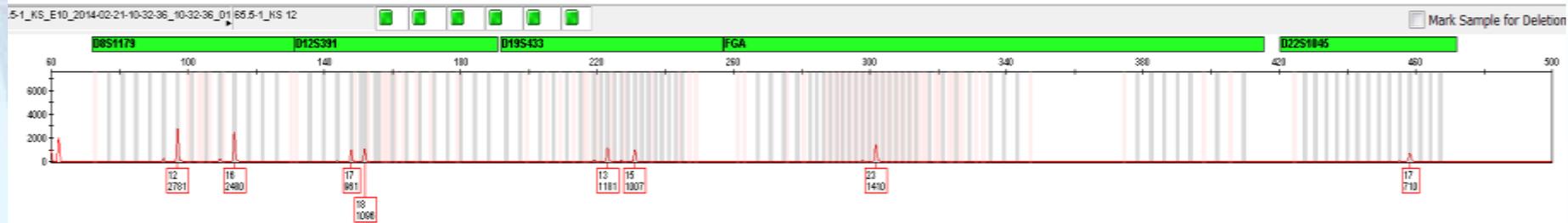
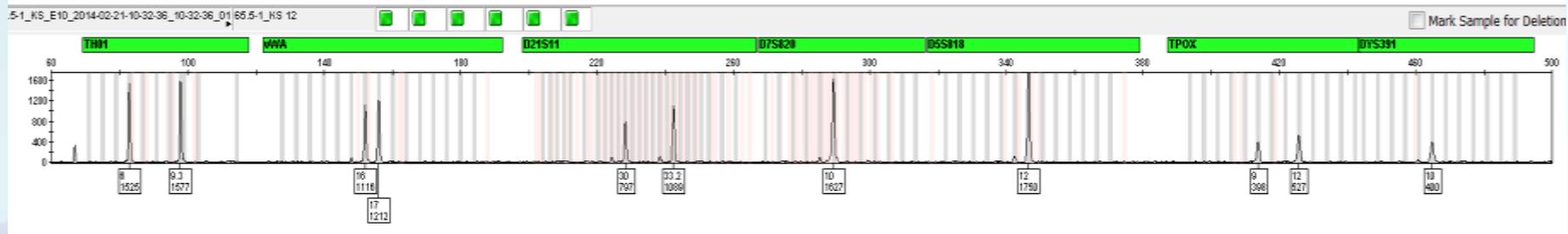
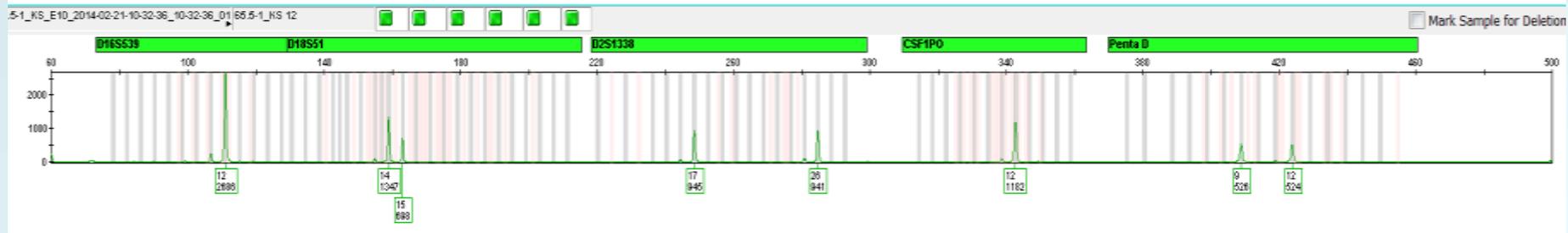
↑
31.25 pg

↑
65.5 pg

↑
125 pg

↑
250 pg

Full profiles at 65.5 picograms



Casework Mixtures

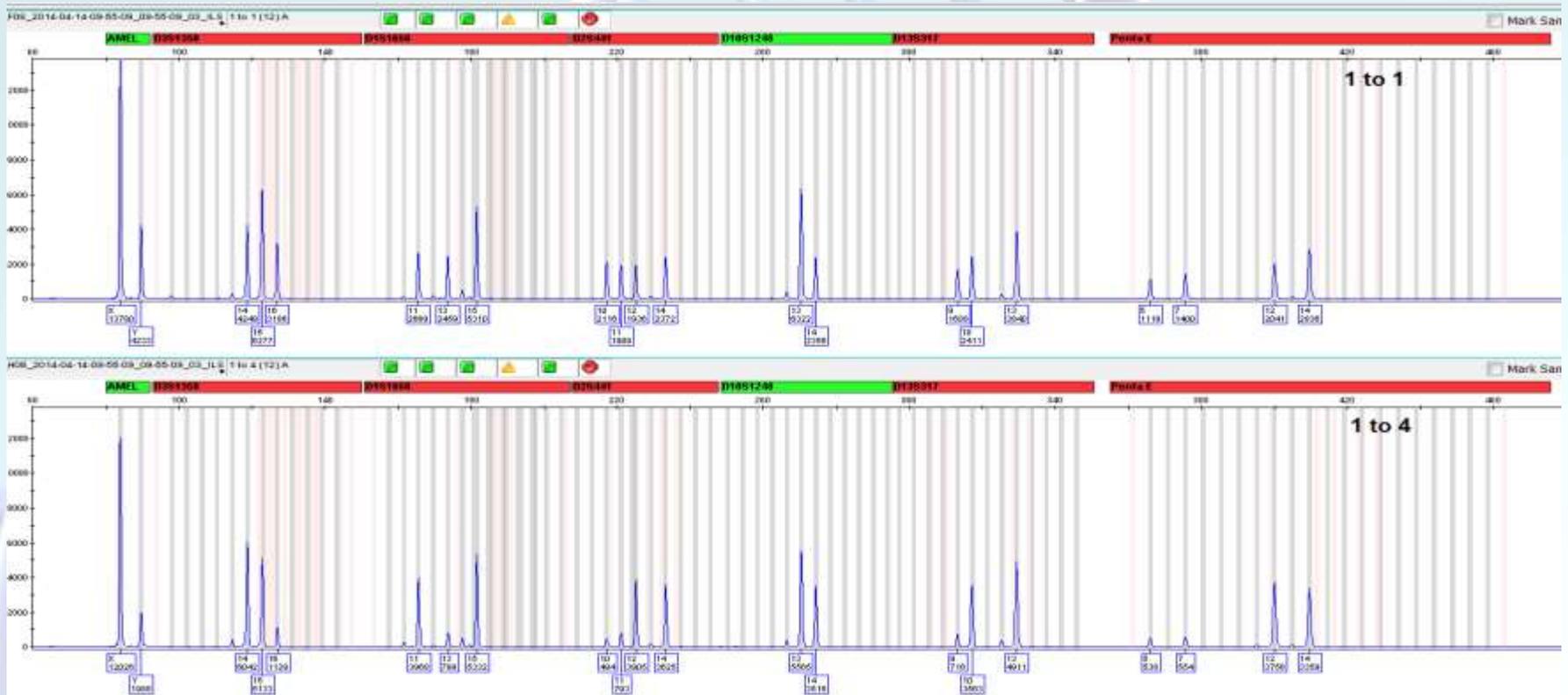
Mixtures were prepared in duplicate using the following ratios with 1.0 ng of Input DNA

- 1:19, 1:9, 1:4, 1:2, 1:1, 2:1, 4:1, 9:1, 19:1 Male:Male
- 1:19, 1:9, 1:4, 1:2, 1:1, 2:1, 4:1, 9:1, 19:1 Female:Male
- Each amplified product was subjected to four injection times (8, 12, 18, and 24 seconds)

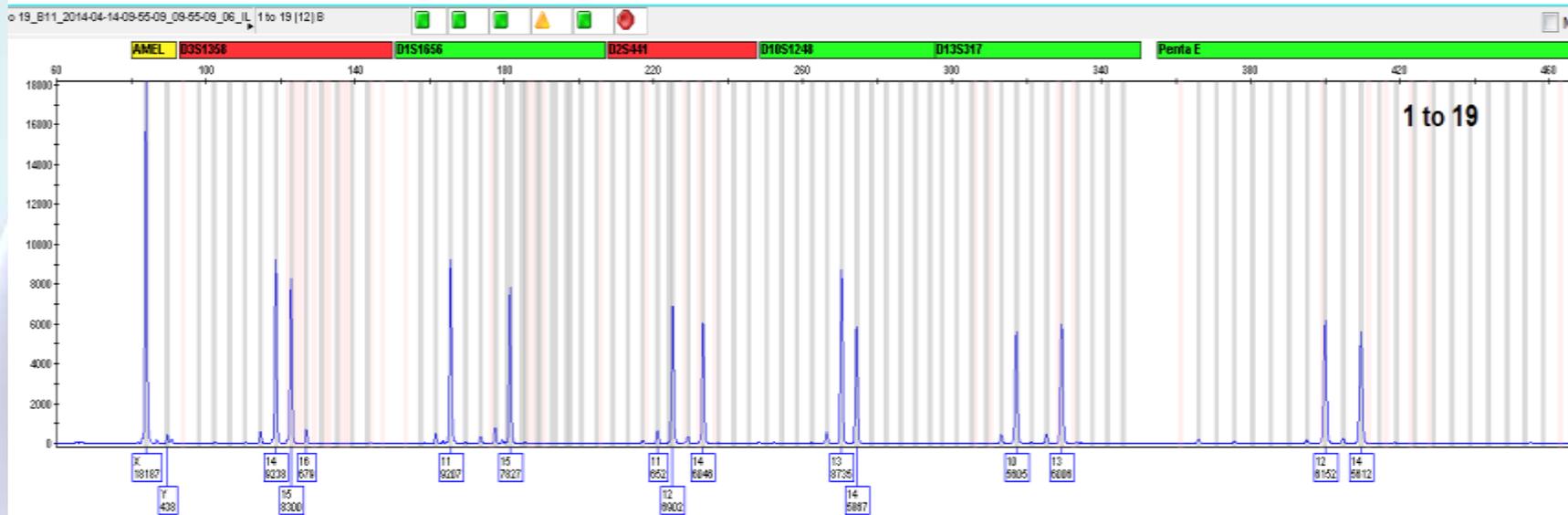
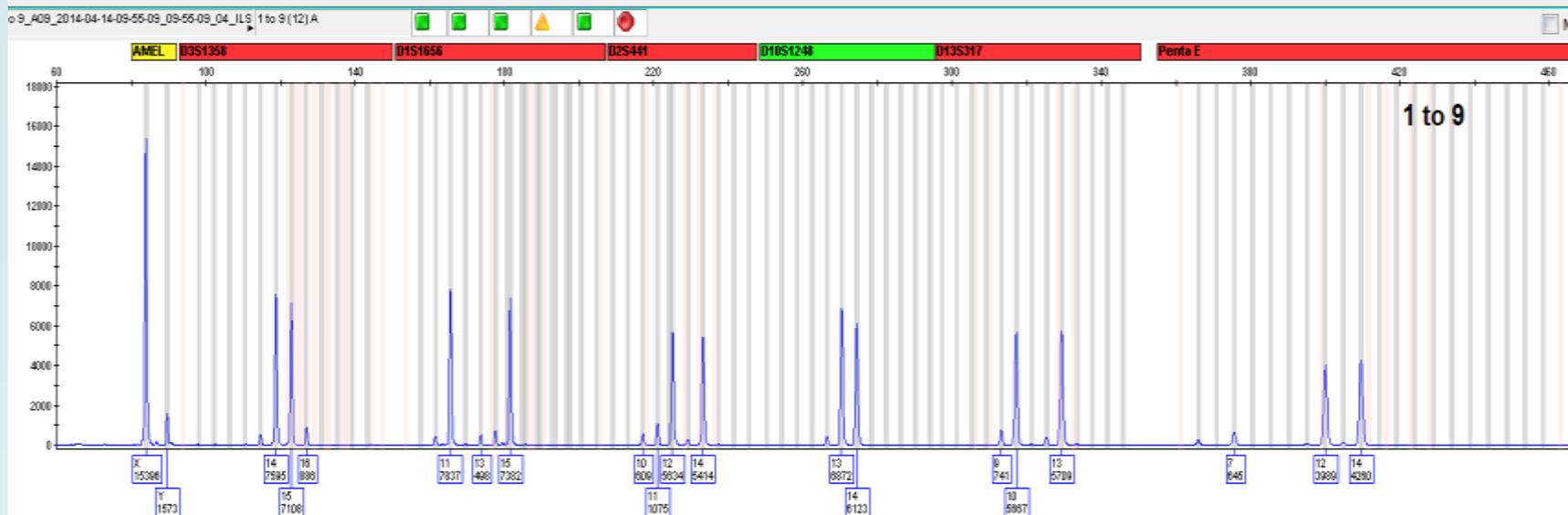
ArmedXpert™ was used to separate the mixtures and handle the data

Casework Mixtures

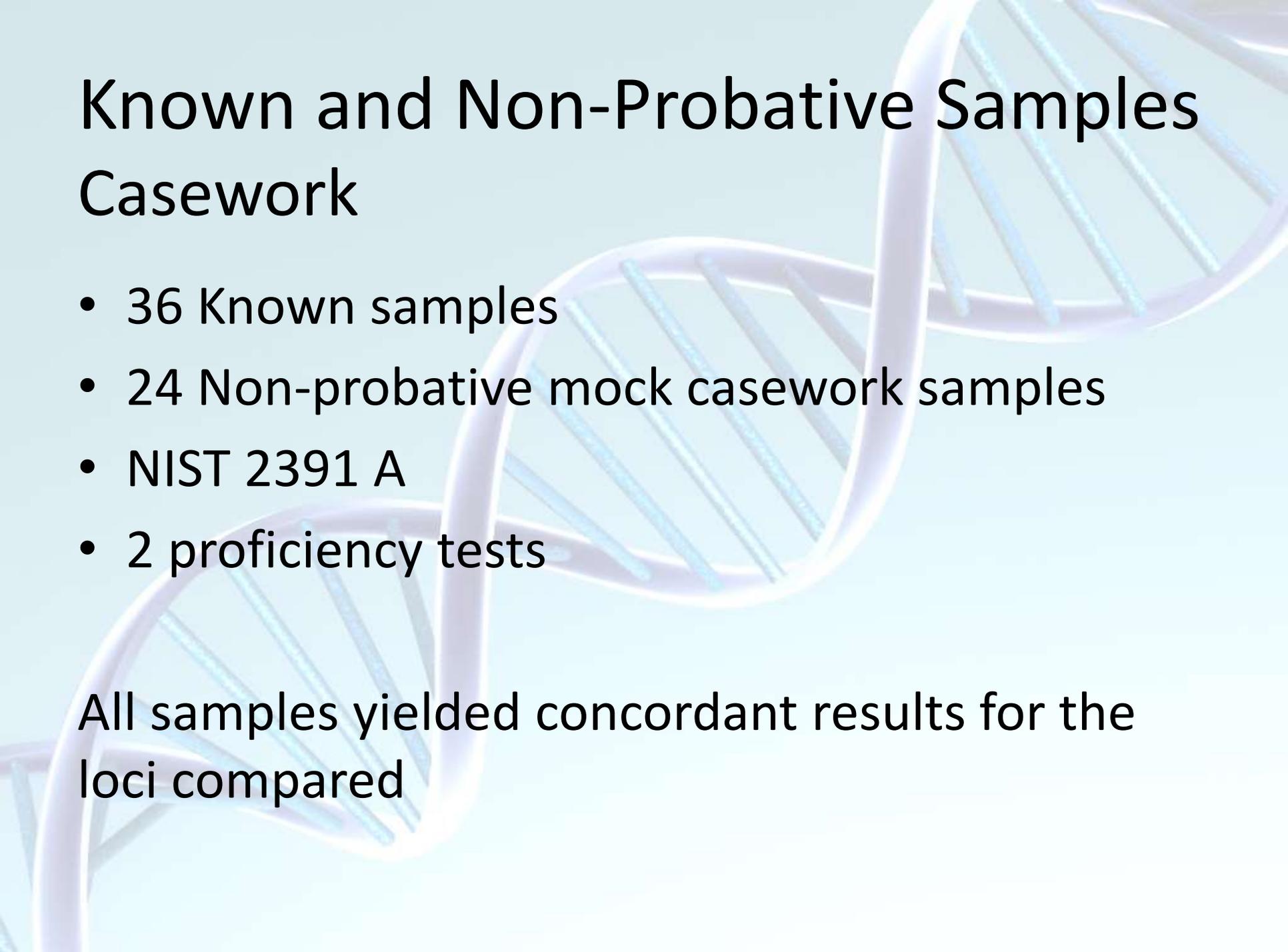
Alleles from the minor contributor were detected in all mixture proportions:



Casework Mixtures



Known and Non-Probative Samples Casework



- 36 Known samples
- 24 Non-probative mock casework samples
- NIST 2391 A
- 2 proficiency tests

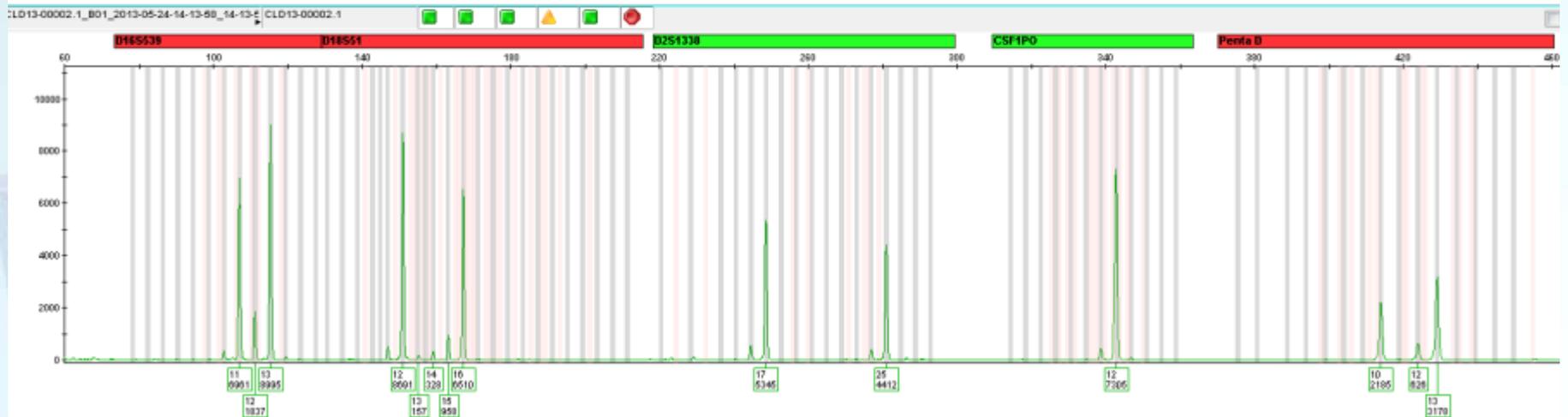
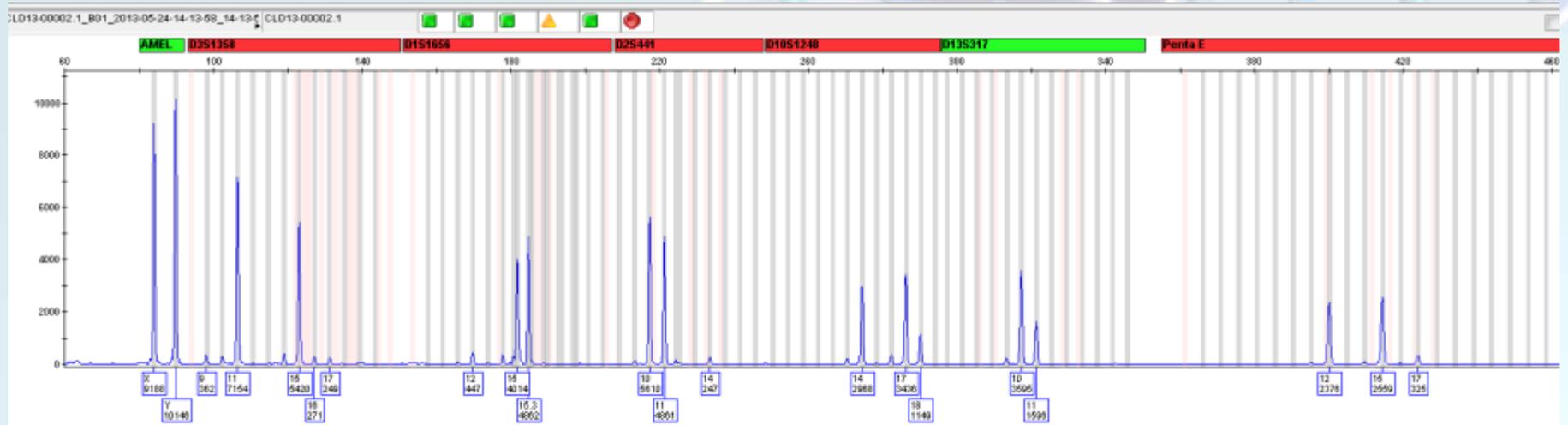
All samples yielded concordant results for the loci compared

Mock Casework

- 24 Non-Probative samples
 - Cigarette butt
 - Baseball cap
 - T-shirt swab
 - Urine swab
 - Feces swab
 - Steering wheel swab (2)
 - Blood swab (8 samples)
 - Swab of a coat hanger
 - Swab of ignition
 - Swab of shoe
 - Swab of a knife handle
 - Semen samples (2)
 - Fingernail scrapings

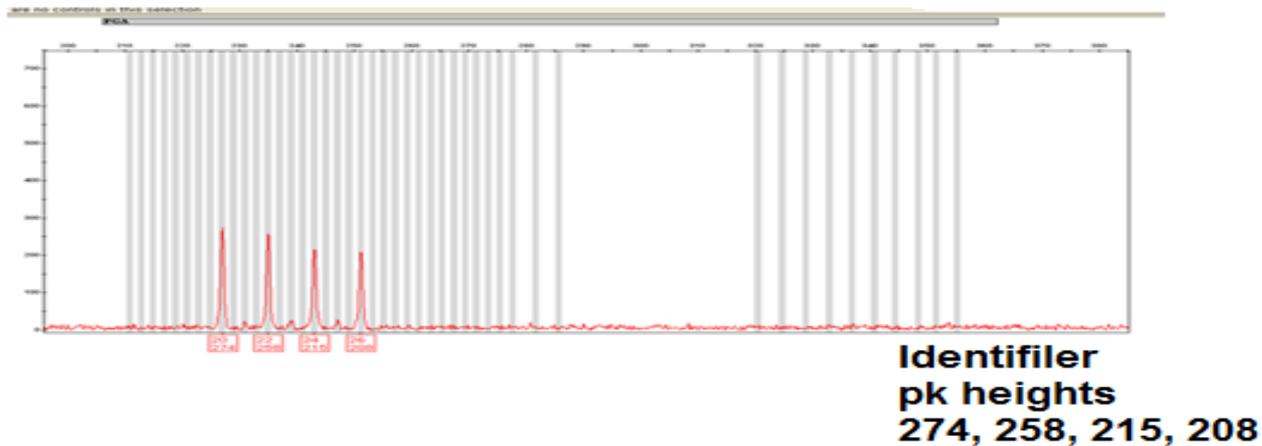
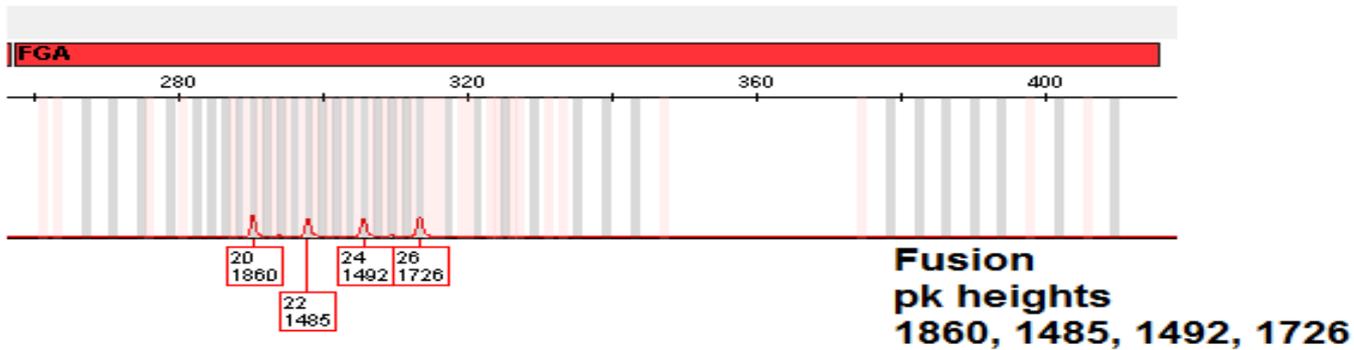
Mock Casework

Baseball cap

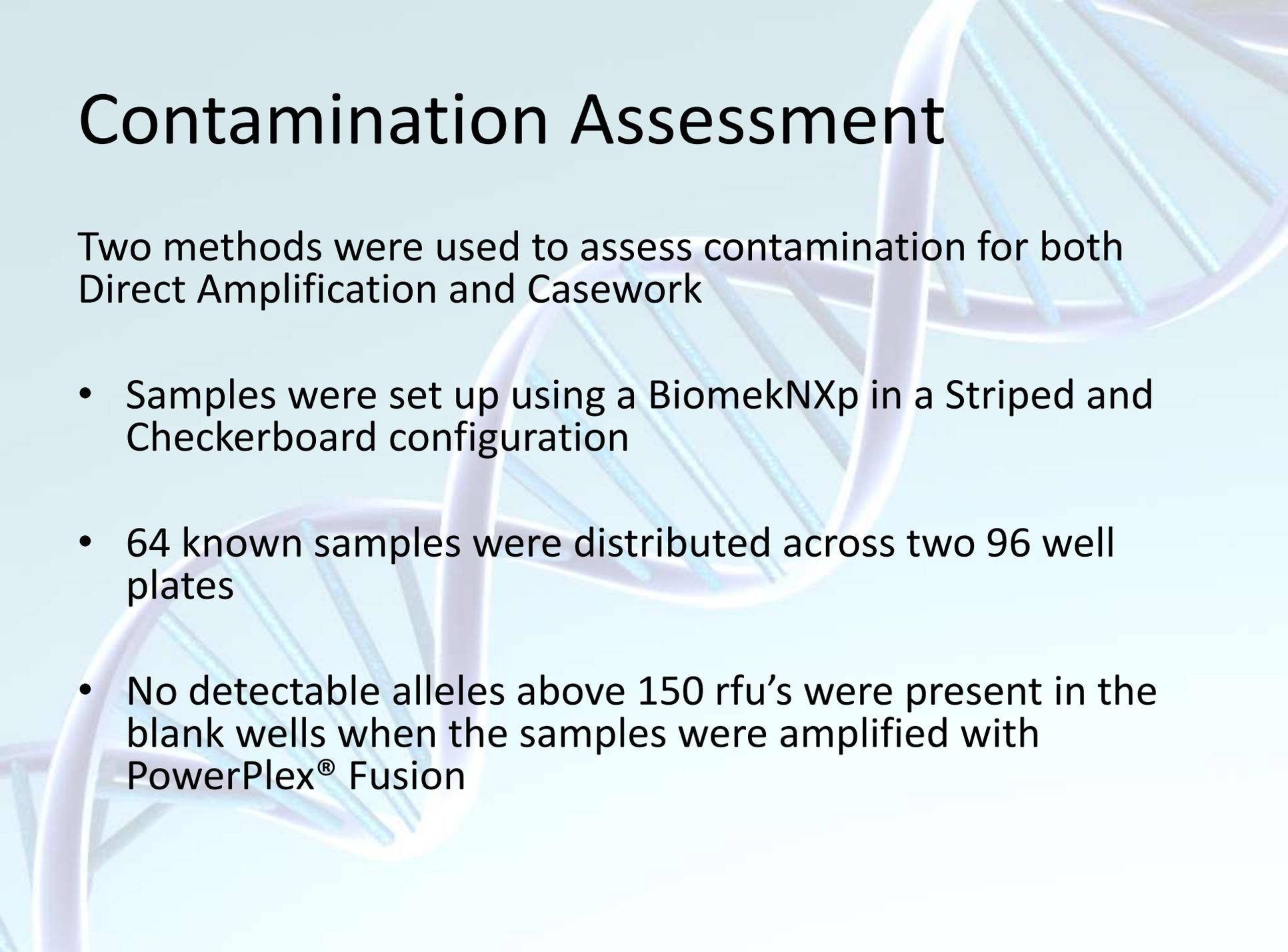


Mock Casework

Comparing AmpF ℓ ISTR $^{\text{®}}$ Identifiler with PowerPlex $^{\text{®}}$ Fusion



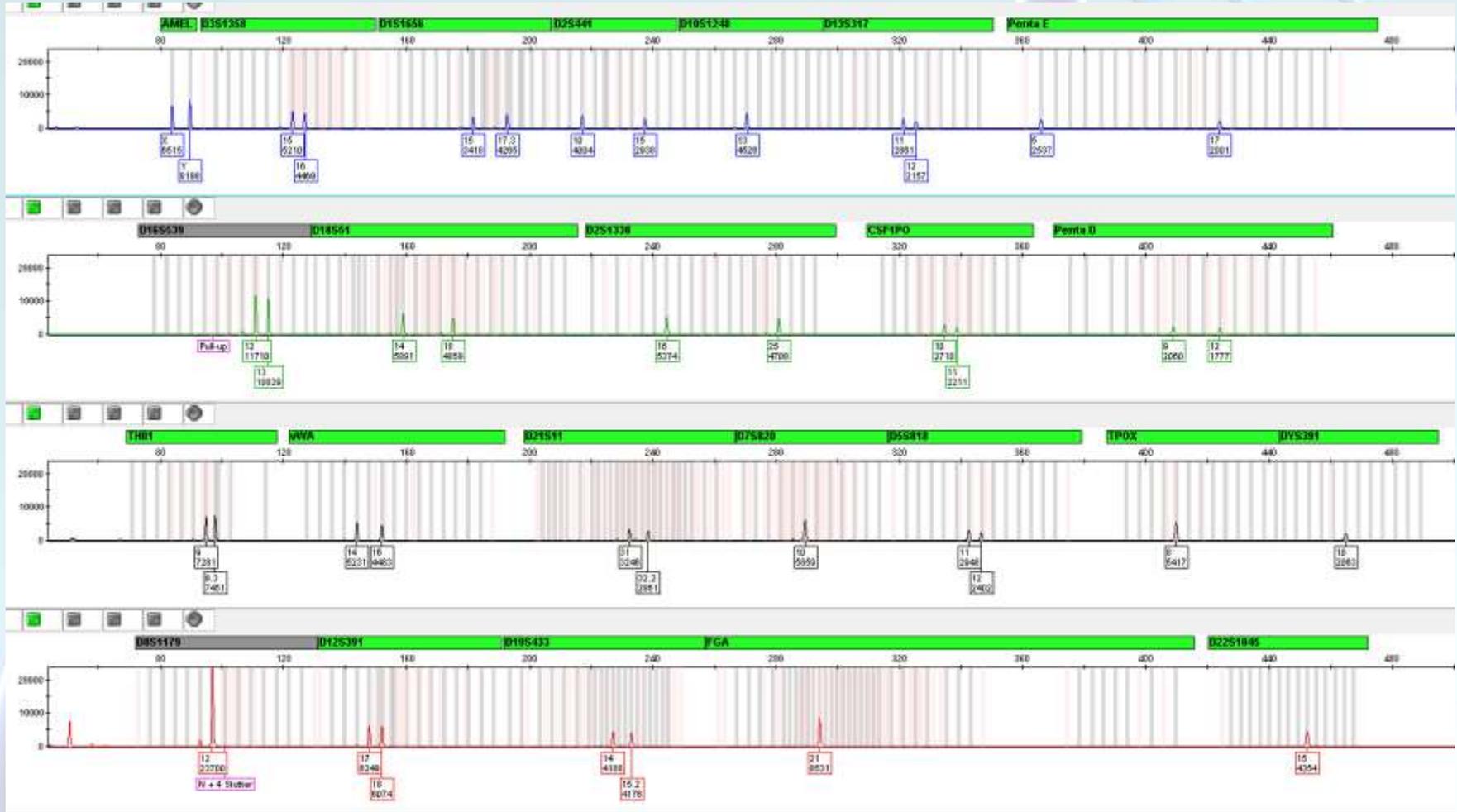
Contamination Assessment



Two methods were used to assess contamination for both Direct Amplification and Casework

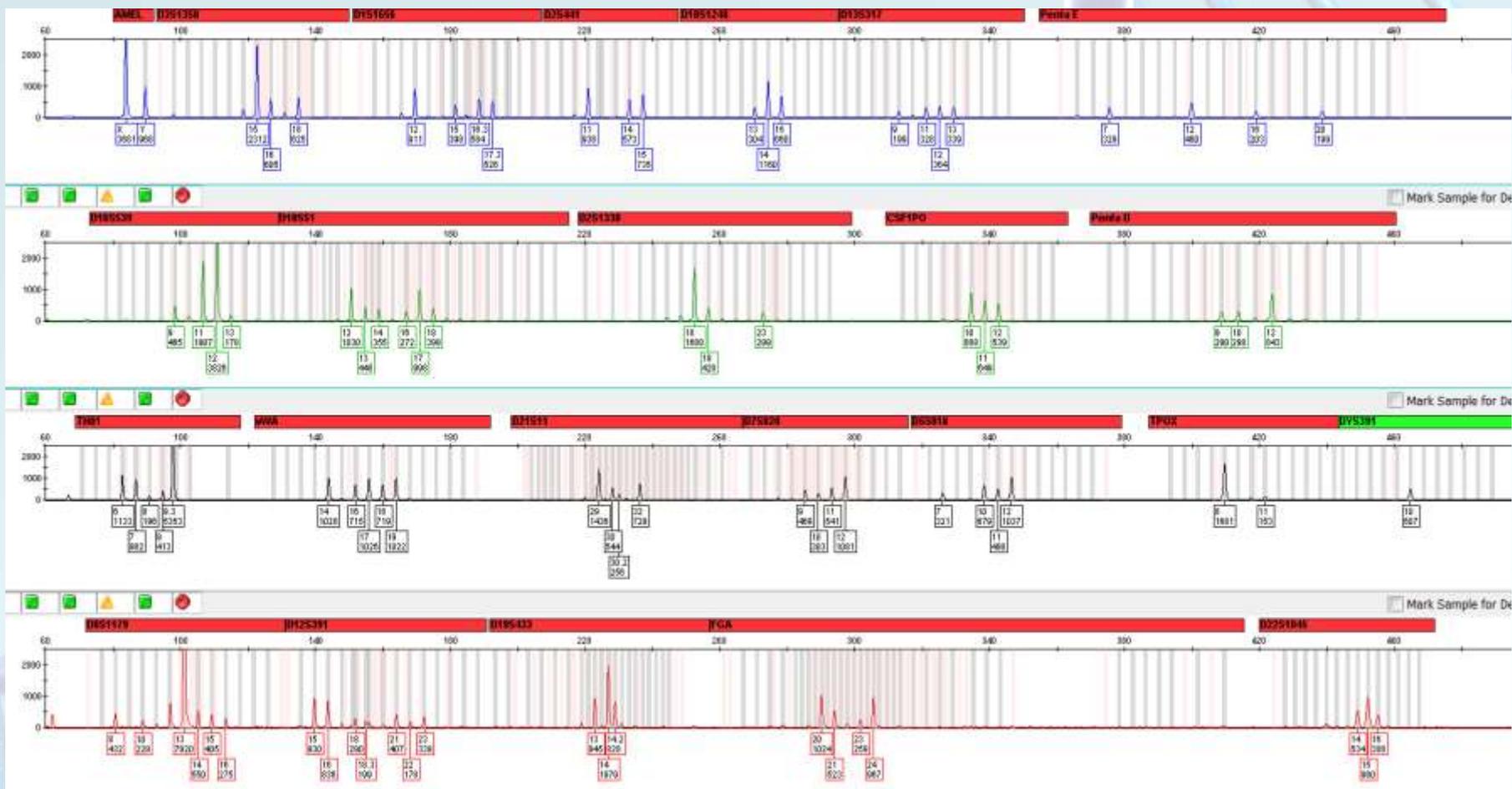
- Samples were set up using a BiomekNXp in a Striped and Checkerboard configuration
- 64 known samples were distributed across two 96 well plates
- No detectable alleles above 150 rfu's were present in the blank wells when the samples were amplified with PowerPlex[®] Fusion

PowerPlex[®] Fusion Casework



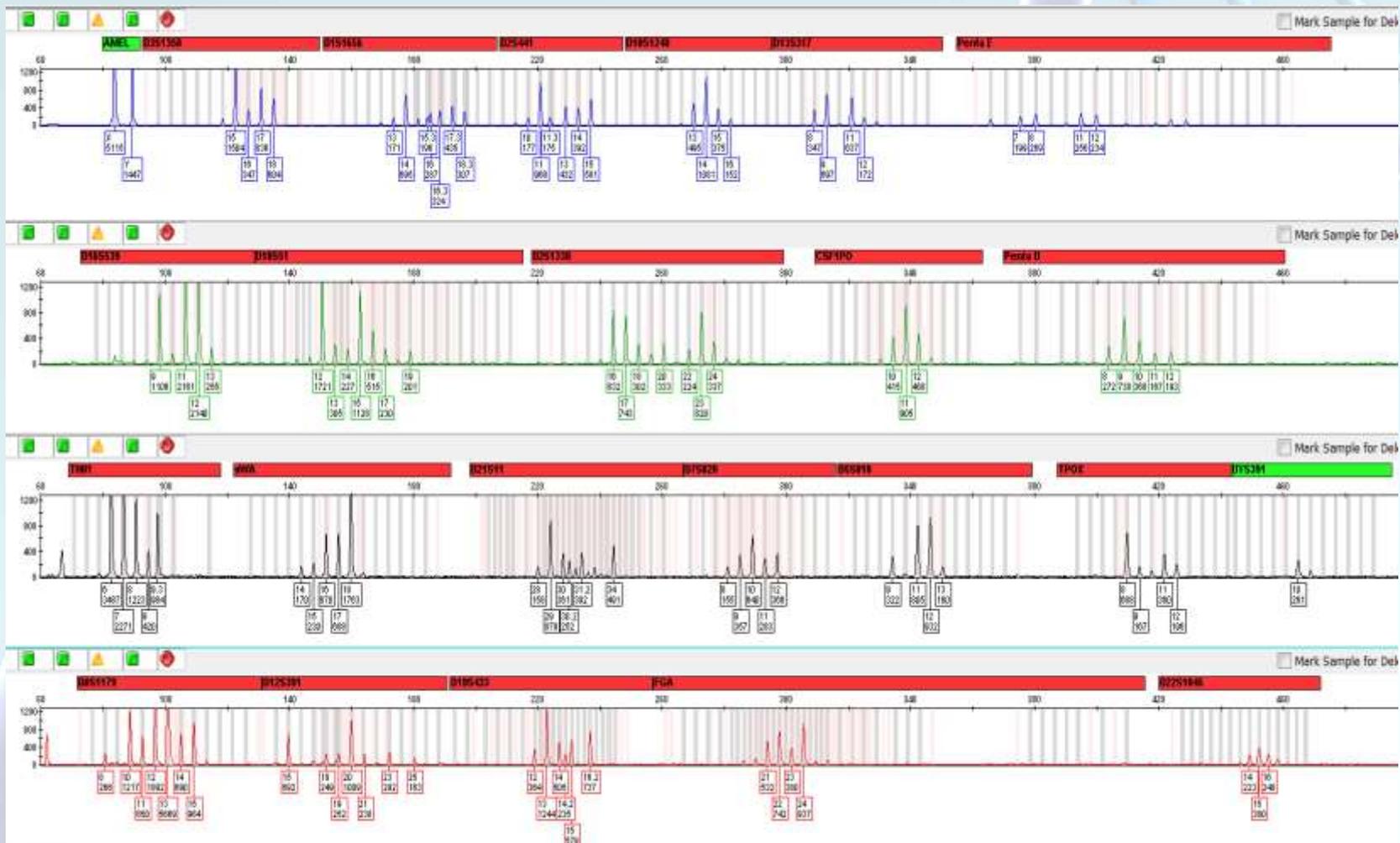
Cigarette Butt

PowerPlex[®] Fusion Casework



Swab from car seat

PowerPlex® Fusion Casework



Swab from a safe door

Acknowledgements

ND Office of Attorney General
Crime Laboratory Division

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