Development of two Novel DNA Analysis methods to Improve Workflow Efficiency for Challenging Forensic Samples

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InnoGenomics
Innovation in Forensic Genetics

The Problem

Degraded samples pose the following challenges in a forensic DNA lab:

- Poor quality and little information on sample quality prior to PCR amplification
- Low quantity
- Low ratio male/female mixture samples
- Inhibitors present
- Longer time to results due to necessary re-processing steps
- Often obtain unusable profiles (inconclusive or no result)

How does a DNA analyst determine whether to continue with typing analysis, which typing test kit to use and how much DNA to add to the amplification reaction to obtain a useful profile in the first pass?

Possible Solutions

1. InnoQuant: Tools to provide additional information on sample quality prior to PCR amplification. These tools must provide:
   - Accurate quantitation values to reduce downstream re-processing
   - Sensitive analysis
   - Reproducible results
   - Ease of platform

2. InnoTyper: Tools to provide usable results from degraded samples where conventional STR analysis is unsuccessful. These tools must provide:
   - Sensitive analysis
   - Highly statistically discriminatory results
   - Ease of platform

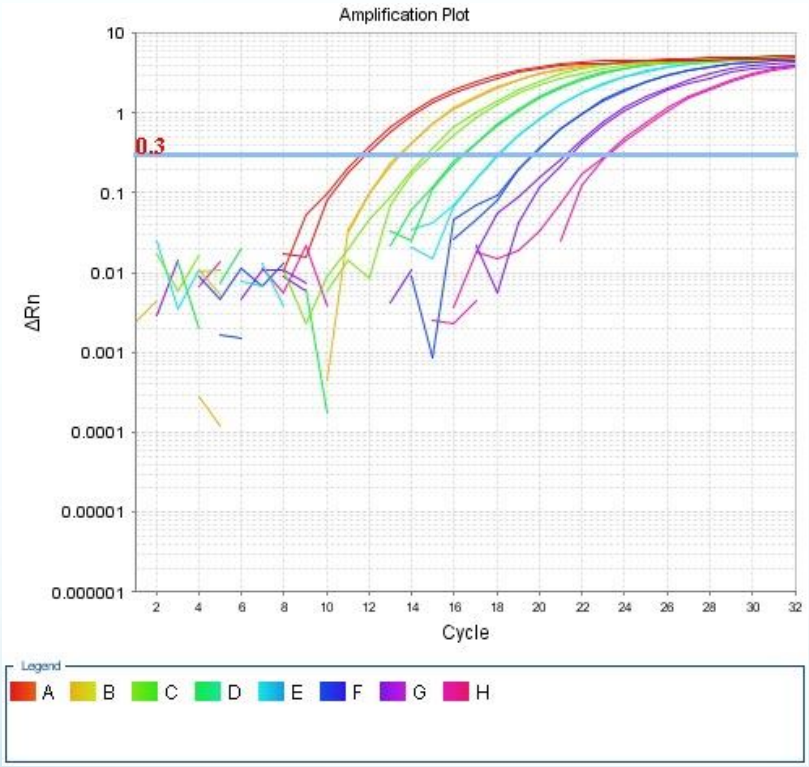
InnoQuant™

Quality and Quantity assessment system

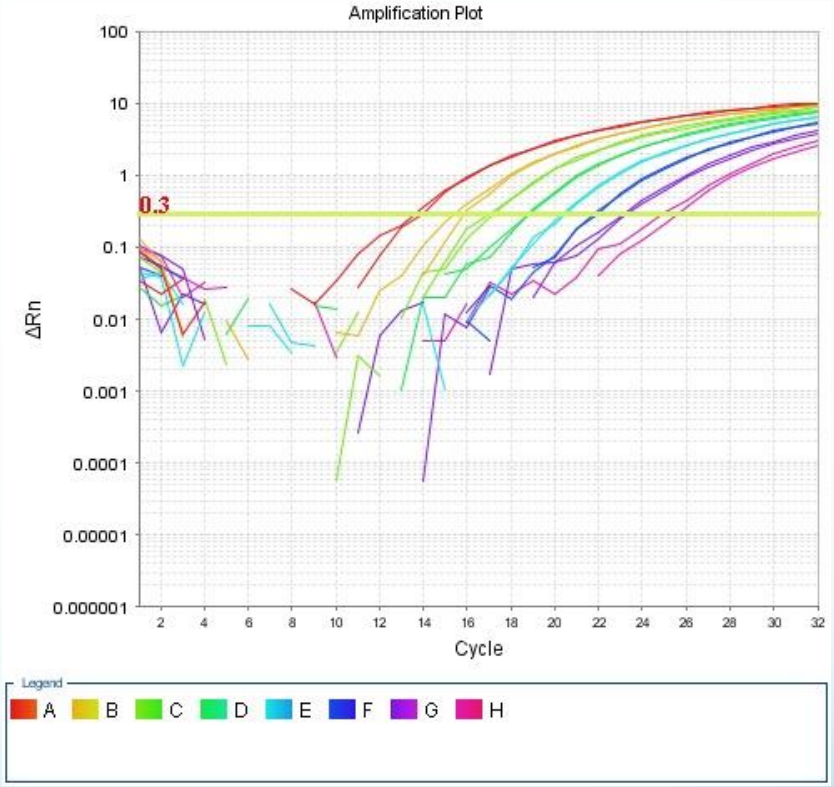
- Three targets qPCR assay:
  - Autosomal target of 80 bp (>1000 copies/genome)
  - Autosomal target of 207 bp (>1000 copies/genome)
  - Synthetic IPC for detection of inhibition
- Provides an additional tool to be used prior to typing: the "Degradation Index" (DI)
  - $DI_{80/207}$ = [short] / [long]
  - $DI_{80/207}$ = 1 means no degradation
  - The higher the DI, the more degradation in sample

Real time PCR amplification plots

Short Target

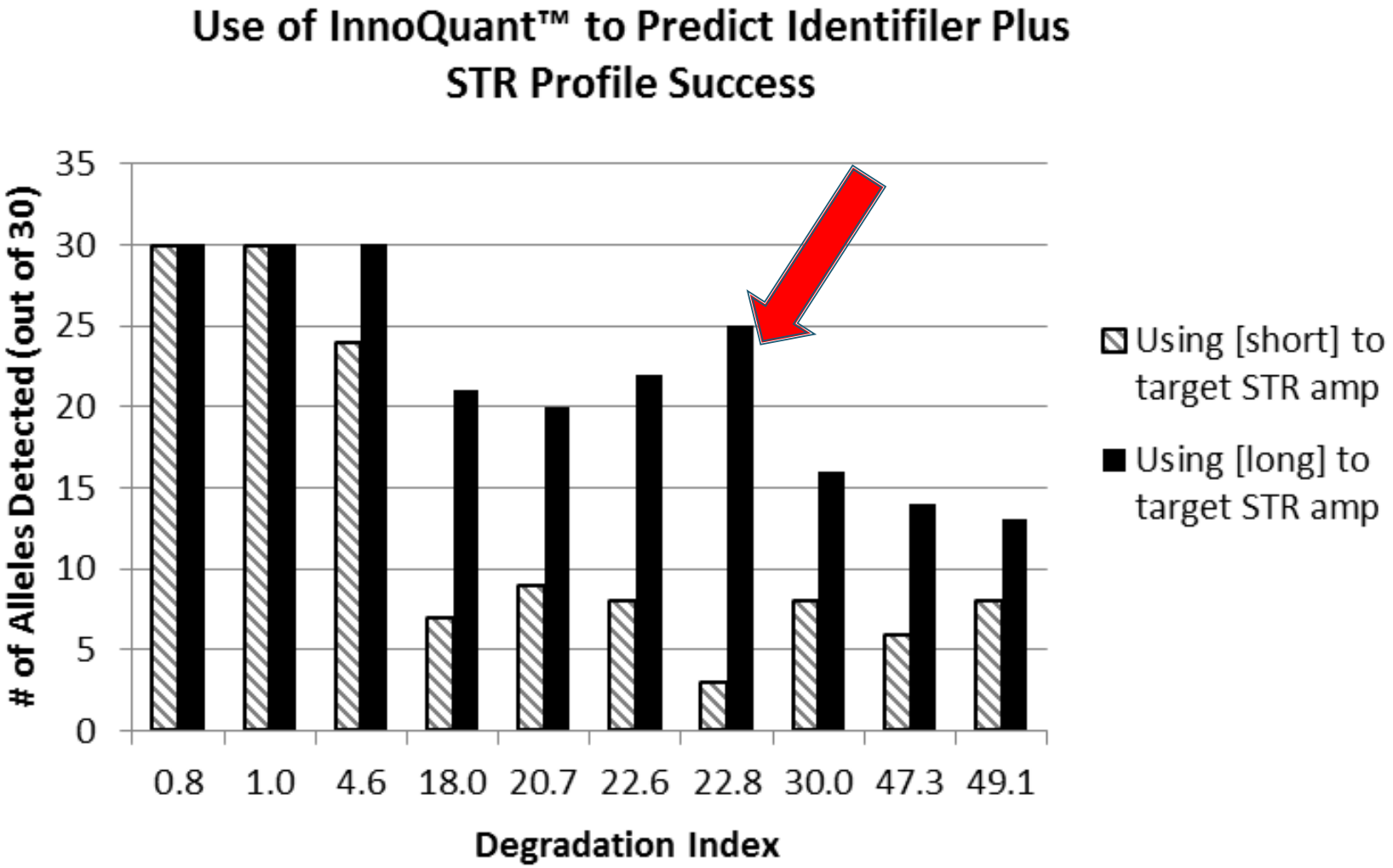


Long Target

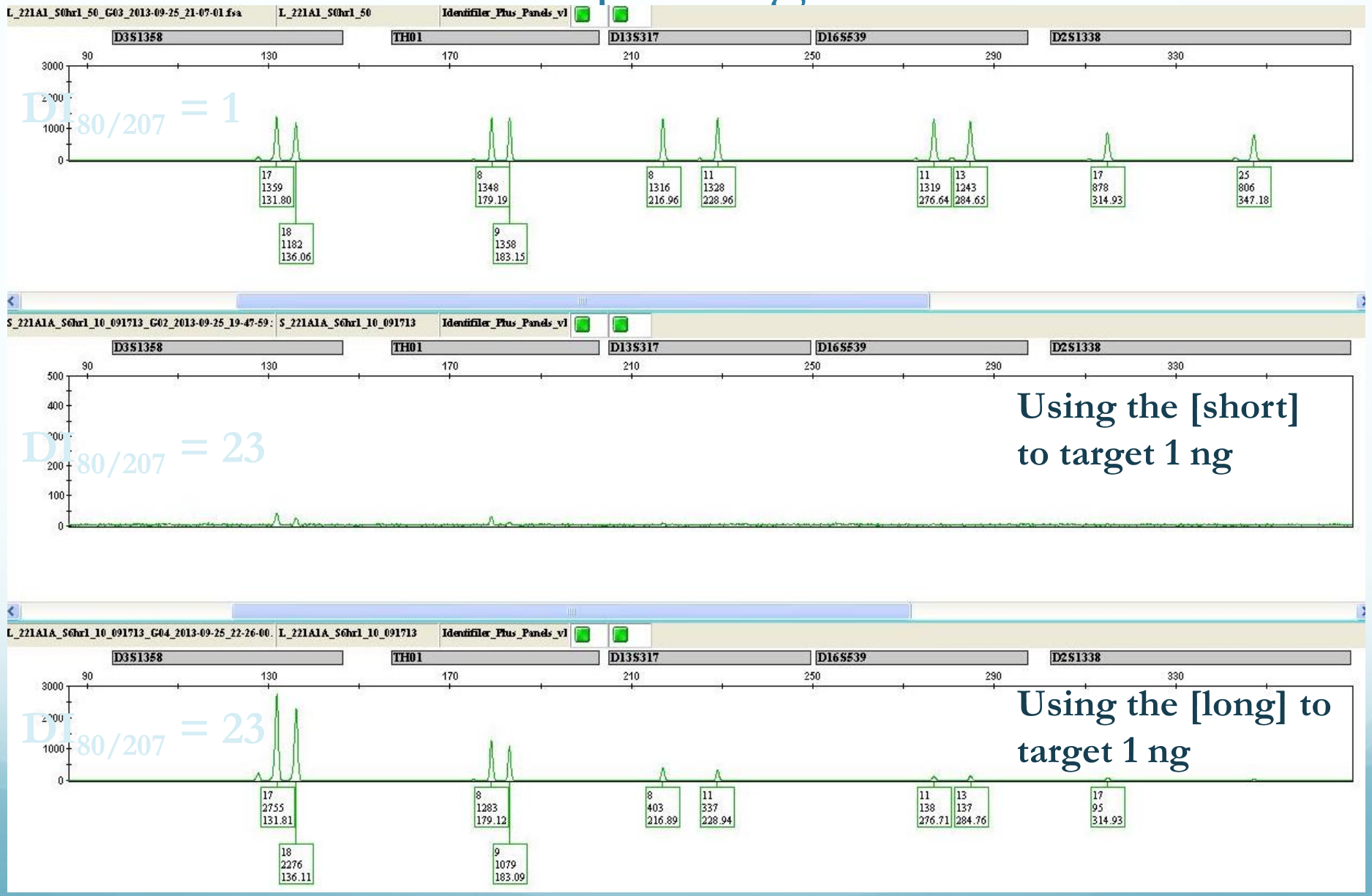


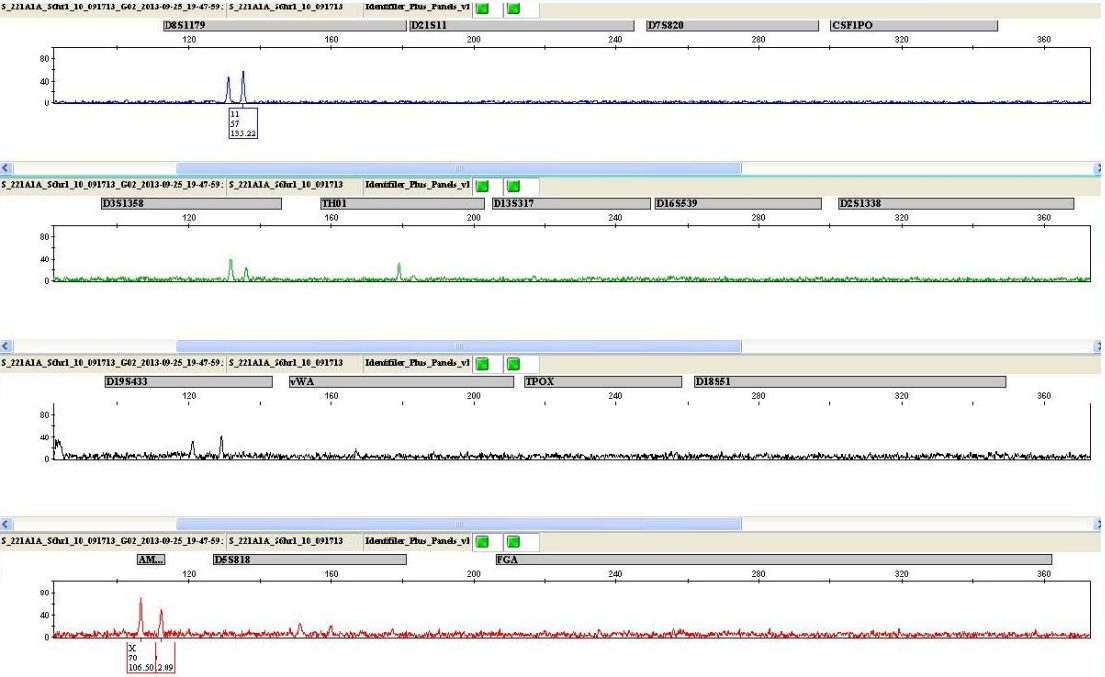
PCR efficiency:	96.689%	98.153%
Slope:	-3.404	-3.367
R2:	0.998	0.996

Figure 10: Degradation Study



IDP Electropherogram: Green



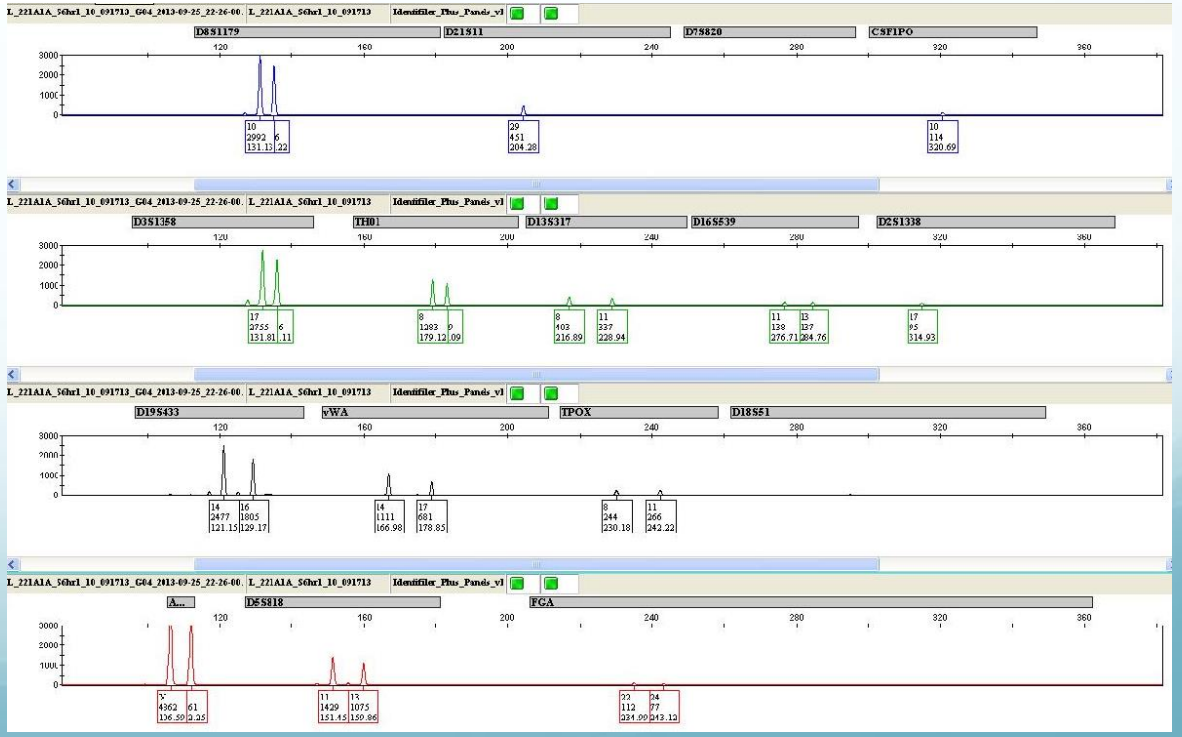


Using the [short] to target IDP amp:

- 3 out of 30 alleles called in $DI_{80/207}$ = 23 sample

Using the [long] to target IDP amp:

- 23 out of 30 alleles called in $DI_{80/207}$ = 23 sample



Advantages of InnoQuant™

- Provides an additional tool, the "Degradation Index" (DI), which can be used to more informatively select the typing system and the amount of DNA used
- Can use current platform (i.e. 7500 with either SDS or HID)
- Highly sensitive: <1 picogram
- Large copy number of selected targets minimizes the effect of variation between individuals, resulting in highly reproducible quantitation values.
- Leads to higher efficiency and higher profile success rates
- Development and Validation studies published FSIG November 2014 issue

Now what...?

- You have an indication of the quantity and quality of your sample (the DI)
- Based on the lab's internal validation studies, a DI range can be determined to proceed with conventional STR analysis. But if sample falls outside this range…
- Sample with a DI > 100 (for example) indicating high degradation can proceed with one of the following:
  1. Stop processing & report as "insufficient quality"
  2. Proceed with MiniFiler (or other miniSTR kits) and get results at a few loci
  3. Proceed with mitochondrial DNA (mtDNA) sequencing analysis
  4. Proceed with NG InnoTyper™ 21 kit with small amplicon sizes using current platforms

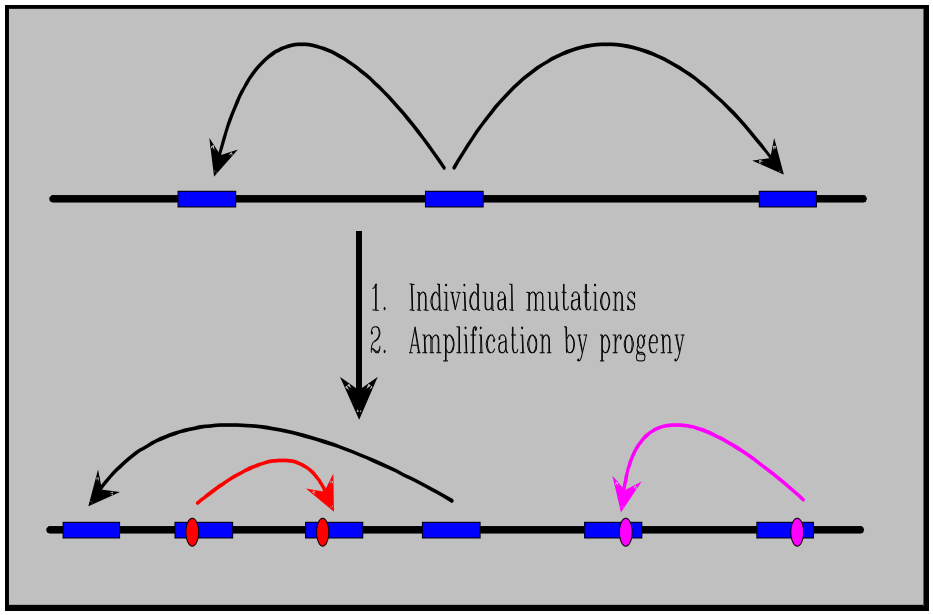
InnoTyper™

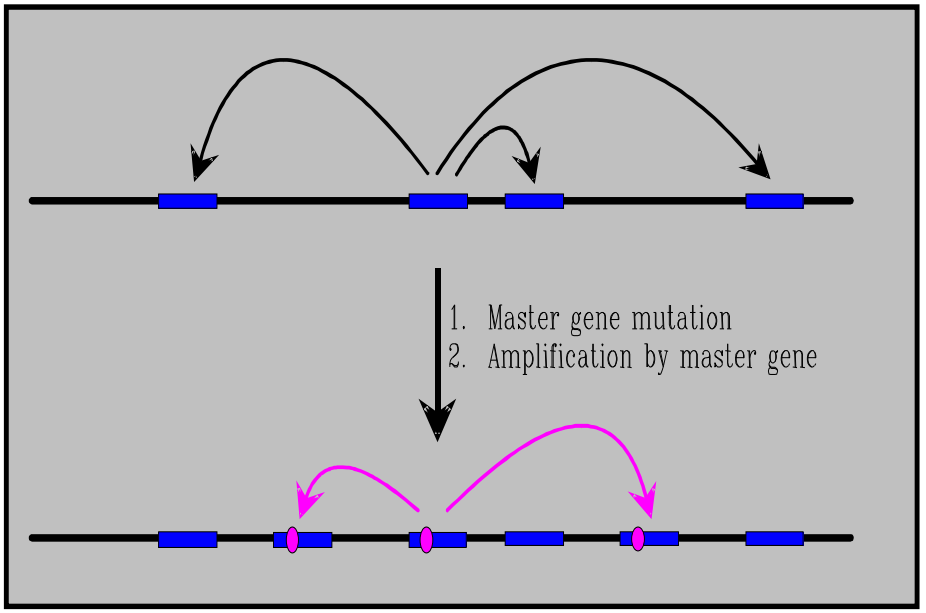
A mobile element based Small Amplicon DNA Typing System

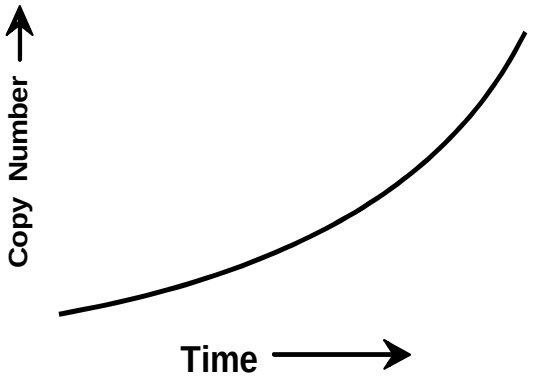
Structure of Alu Element

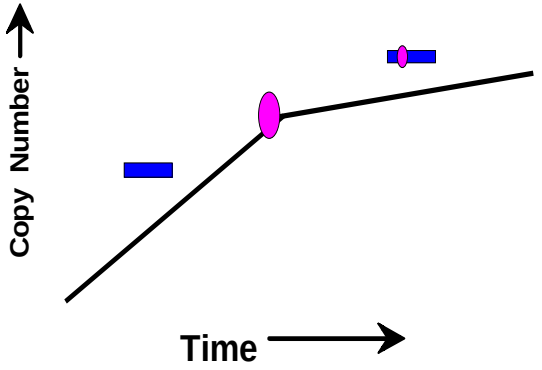
- 300 bp long
- RNA polymerase III transcribed
- 3' oligo dA-rich tail
- 500,000 copies in human genome
- most amplification 40 mya
- similar copy # in Human and other primates
- dimer-like structure
- poorly transcribed

Transposon vs. Master Gene Models

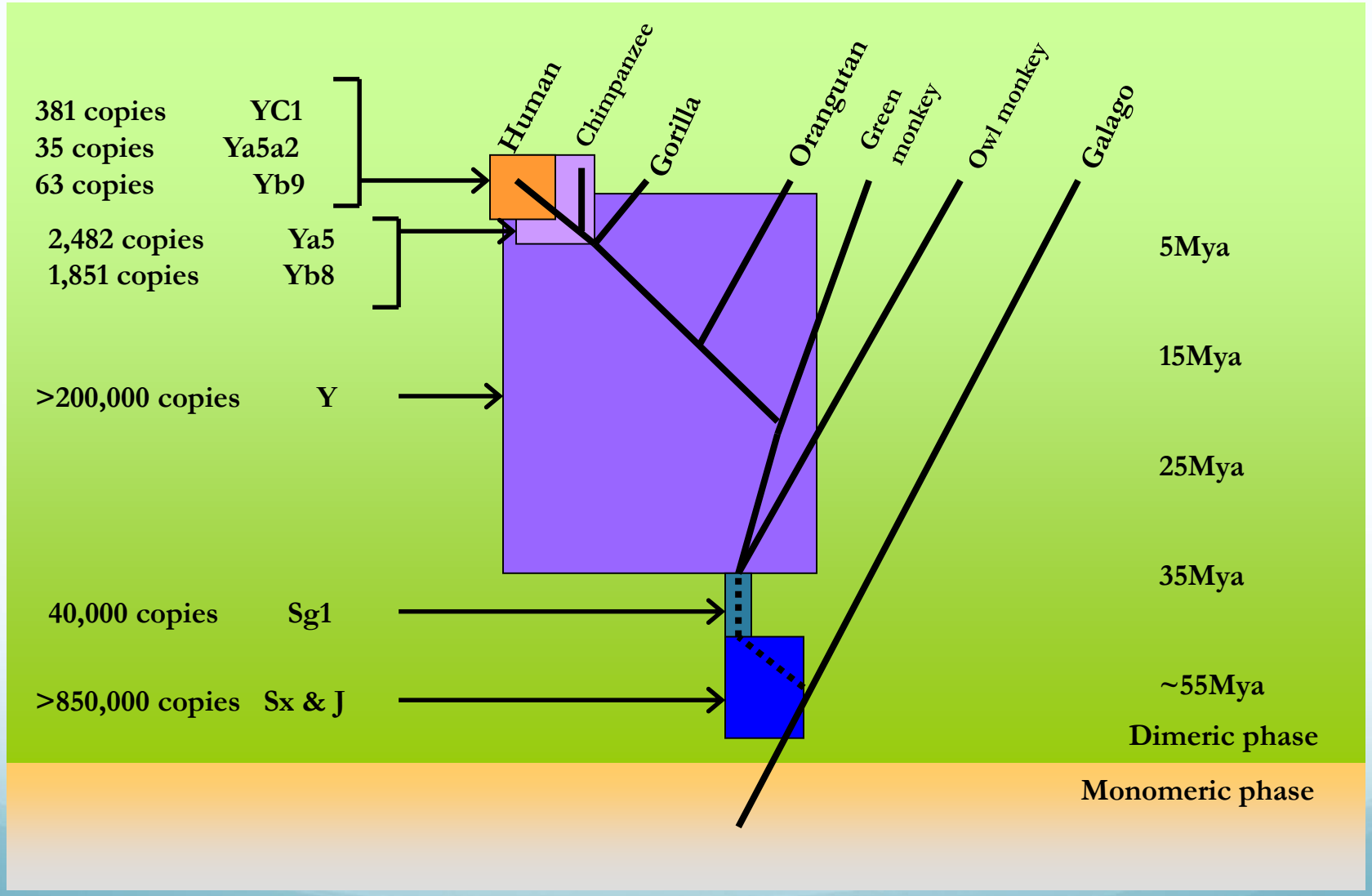








Mobile Elements

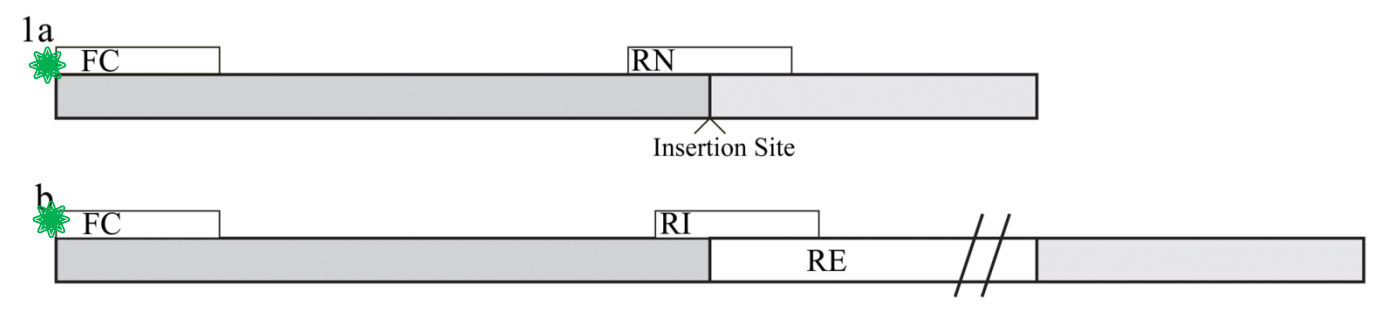


Batzer and Deininger (2002) *Nature Genetics*

Properties of Mobile Element Insertions

1. Stable polymorphisms that are not deleted
2. Known ancestral state
3. Identical by descent
4. Population specific alleles
5. Neutral genetic loci
6. Parallel independent insertion is essentially zero

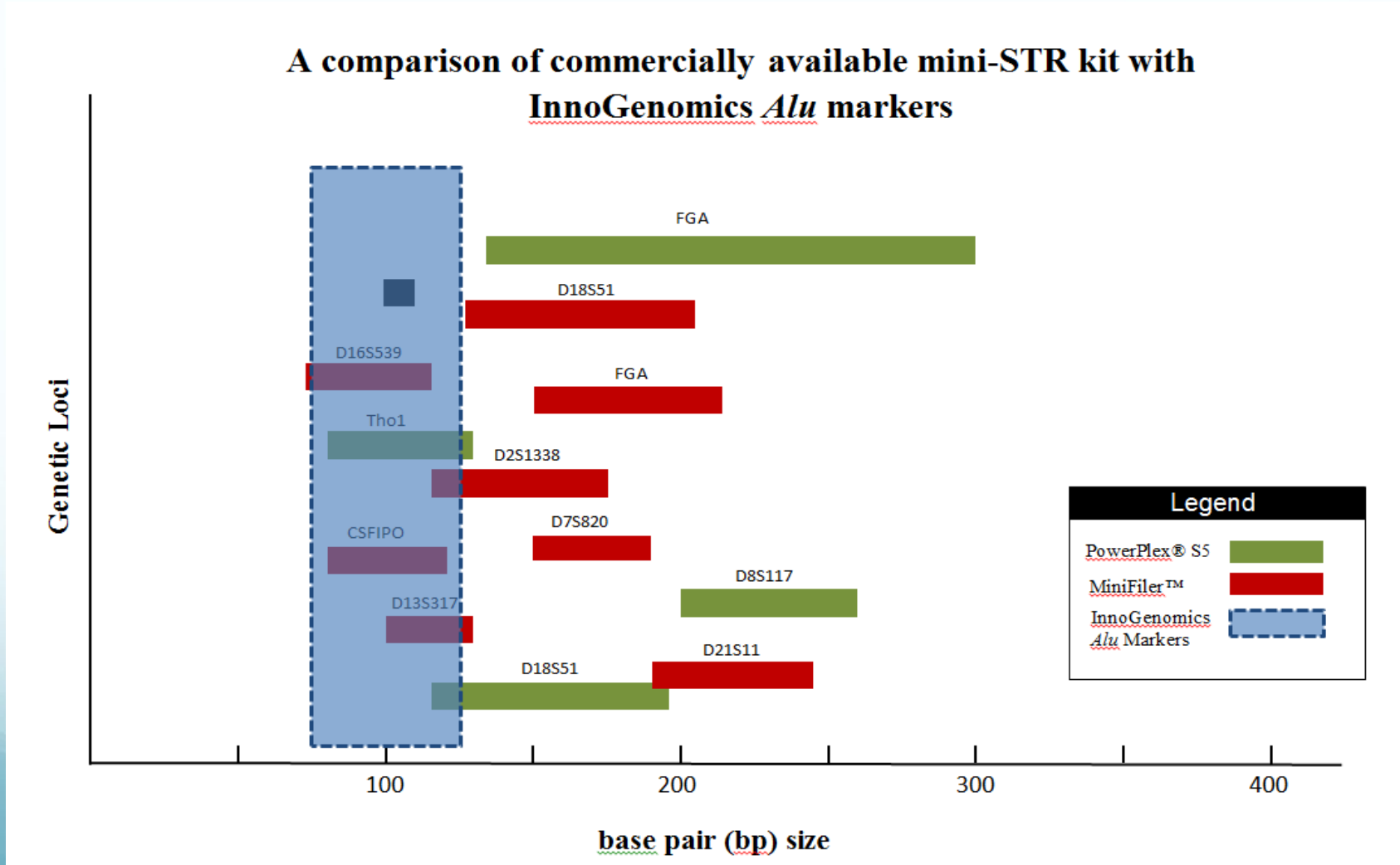
Labeled (common) forward primer and unlabeled reverse



InnoTyper-21

- 20 markers + Amelogenin multiplex
- System amplifies *Alu* and *LINE* sequences less than 125 bp
- This system provides:
  - High sensitivity
  - High tolerance to degradation & inhibition
  - High discrimination power (approx. 1 in 100 million)

BP Size Comparison of mini-STR kits with InnoGenomics markers



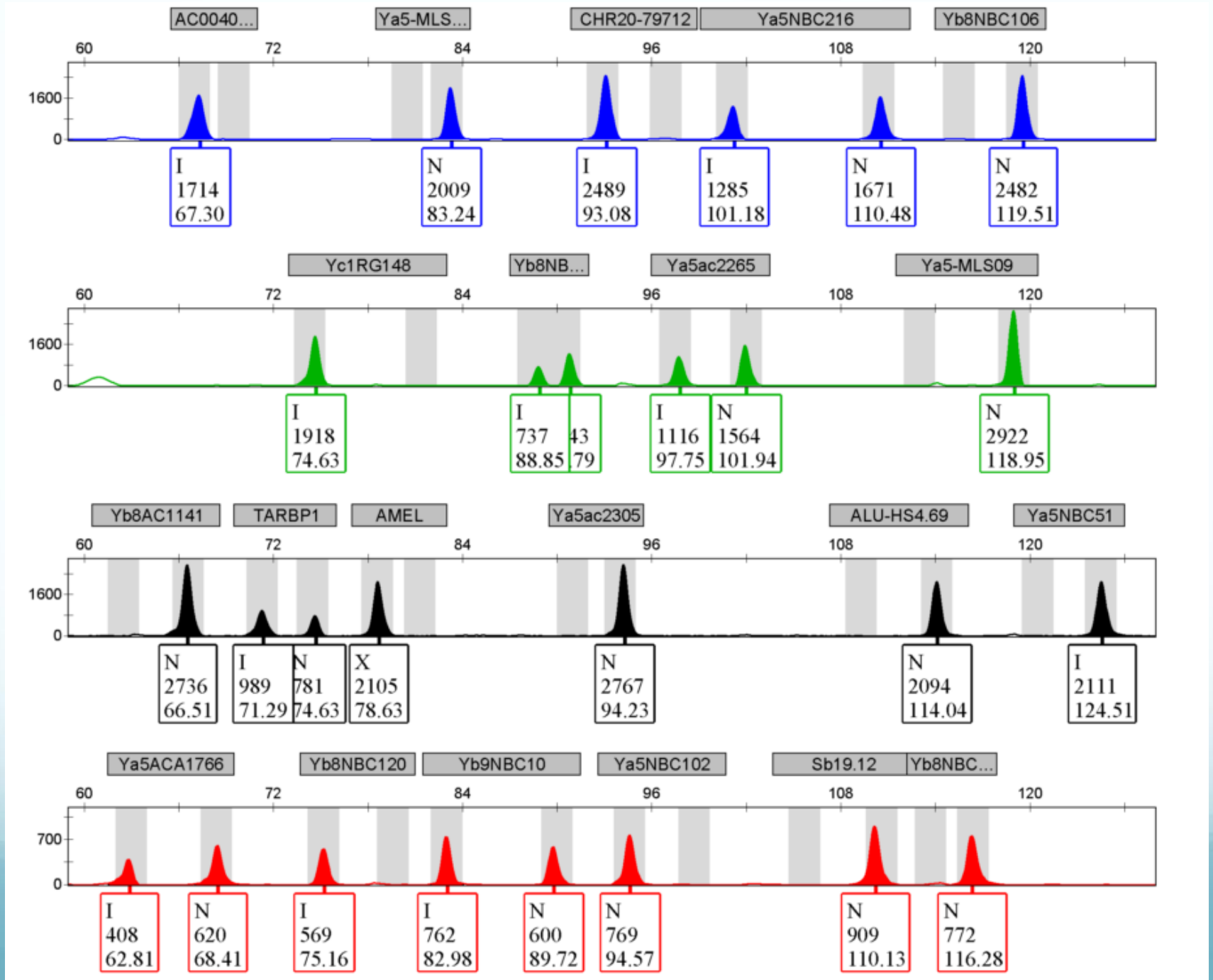
Database Samples

- Four US populations: Caucasian (94), African American(90), Hispanic (92), Asian (90)
- Additional Caucasian and African American:

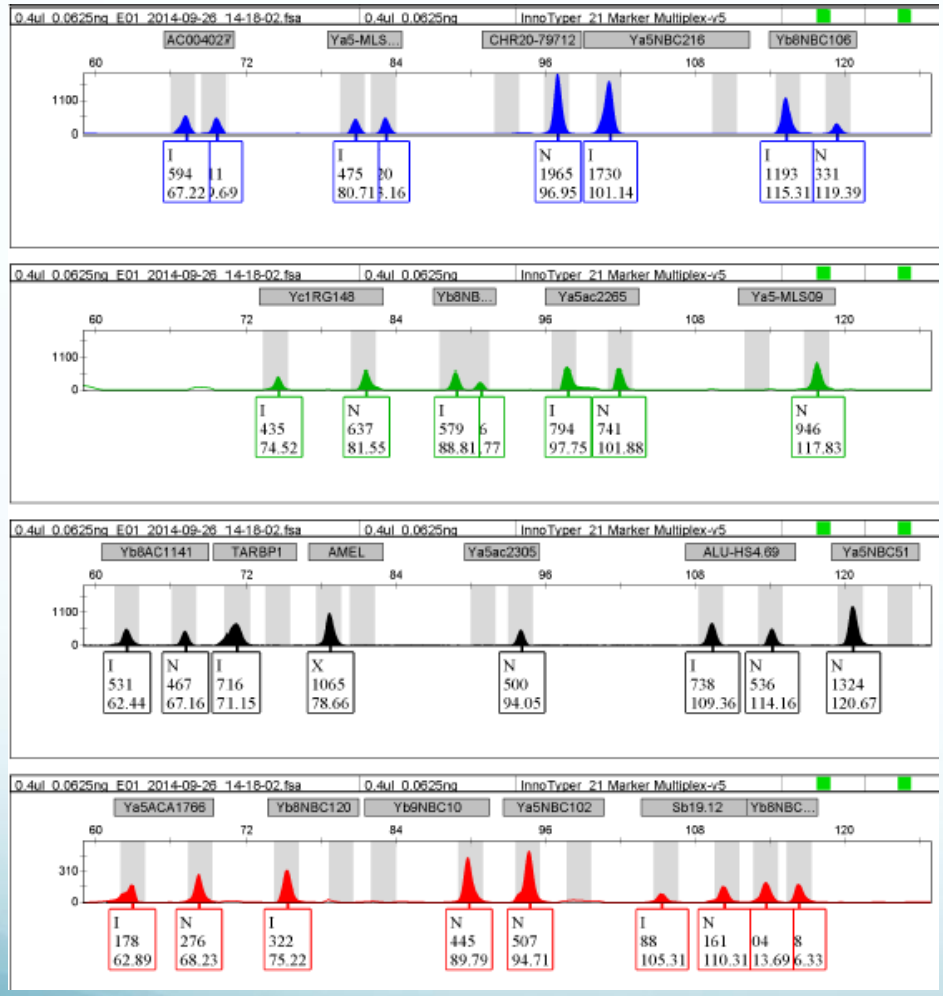
  100 Anonymous Paternity Trios (~200 M and AF samples) with STR data and known Exclusion and Inclusion results.

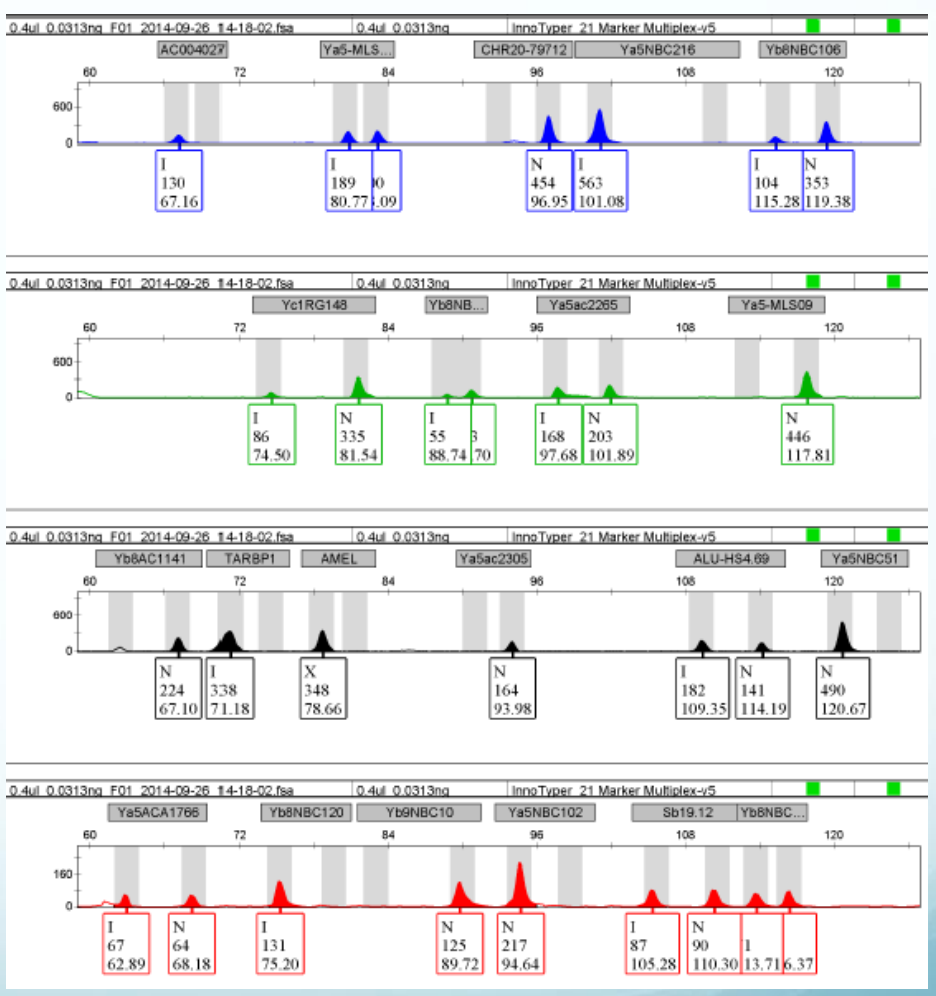
- Environmentally degraded:

Swab samples left at >90˚F in Louisiana for >5 years


AC0040...
Ya5-MLS...
CHR20-79712
Ya5NBC216
Yb8NBC106
60
72
84
96
108
120
1600
0
I 1714 67.30
N 2009 83.24
I 2489 93.08
I 1285 101.18
N 1671 110.48
N 2482 119.51
Yc1RG148
Yb8NB...
Ya5ac2265
Ya5-MLS09
60
72
84
96
108
120
1600
0
I 1918 74.63
I 737 88.85
43 79
I 1116 97.75
N 1564 101.94
N 2922 118.95
Yb8AC1141
TARBP1
AMEL
Ya5ac2305
ALU-HS4.69
Ya5NBC51
60
72
84
96
108
120
1600
0
N 2736 66.51
I 989 71.29
N 781 74.63
X 2105 78.63
N 2767 94.23
N 2094 114.04
I 2111 124.51
Ya5ACA1766
Yb8NBC120
Yb9NBC10
Ya5NBC102
Sb19.12
Yb8NBC...
60
72
84
96
108
120
700
0
I 408 62.81
N 620 68.41
I 569 75.16
I 762 82.98
N 600 89.72
N 769 94.57
N 909 110.13
N 772 116.28

Sensitivity 100 pg and 50pg



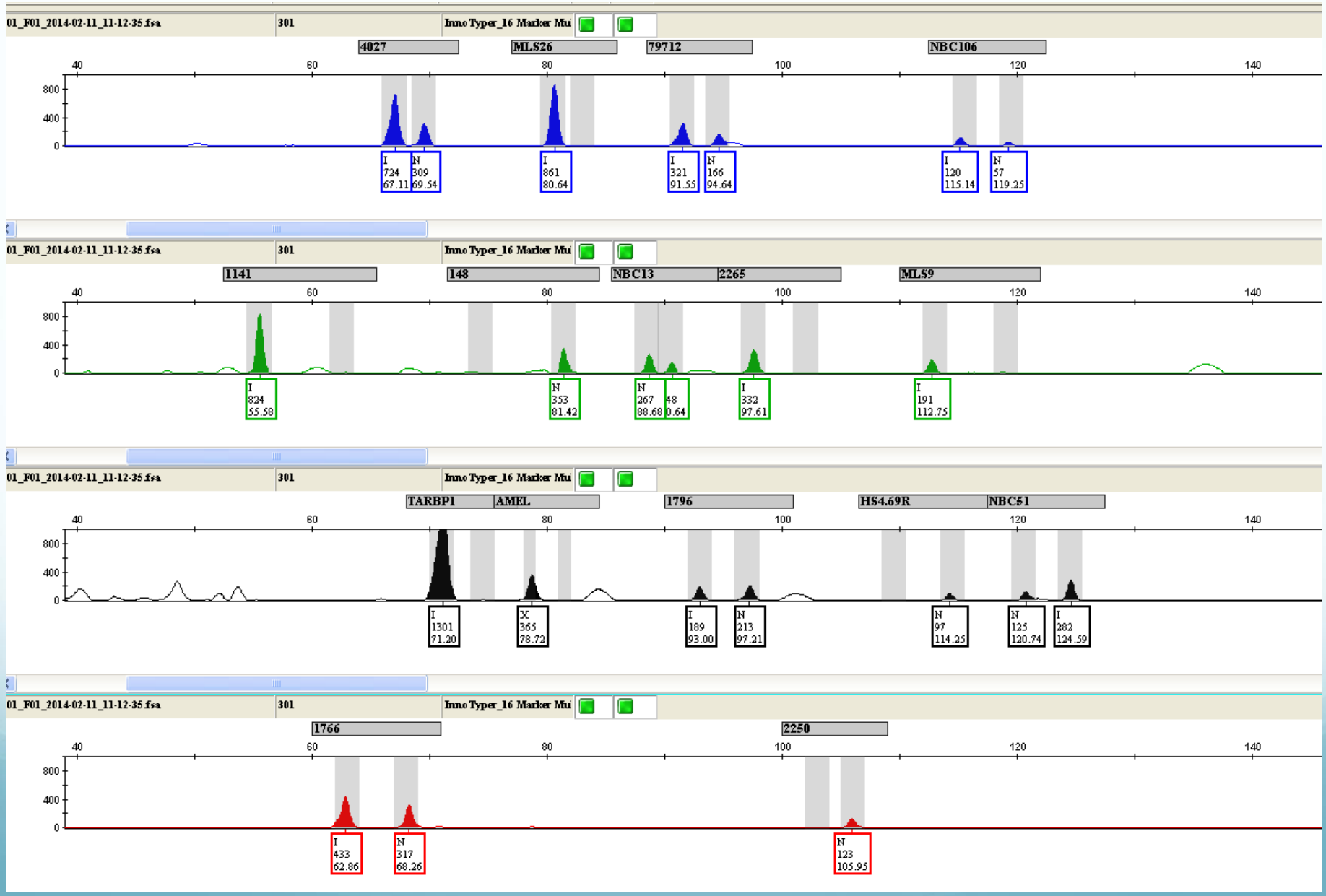


Average heterozygous peak heights:

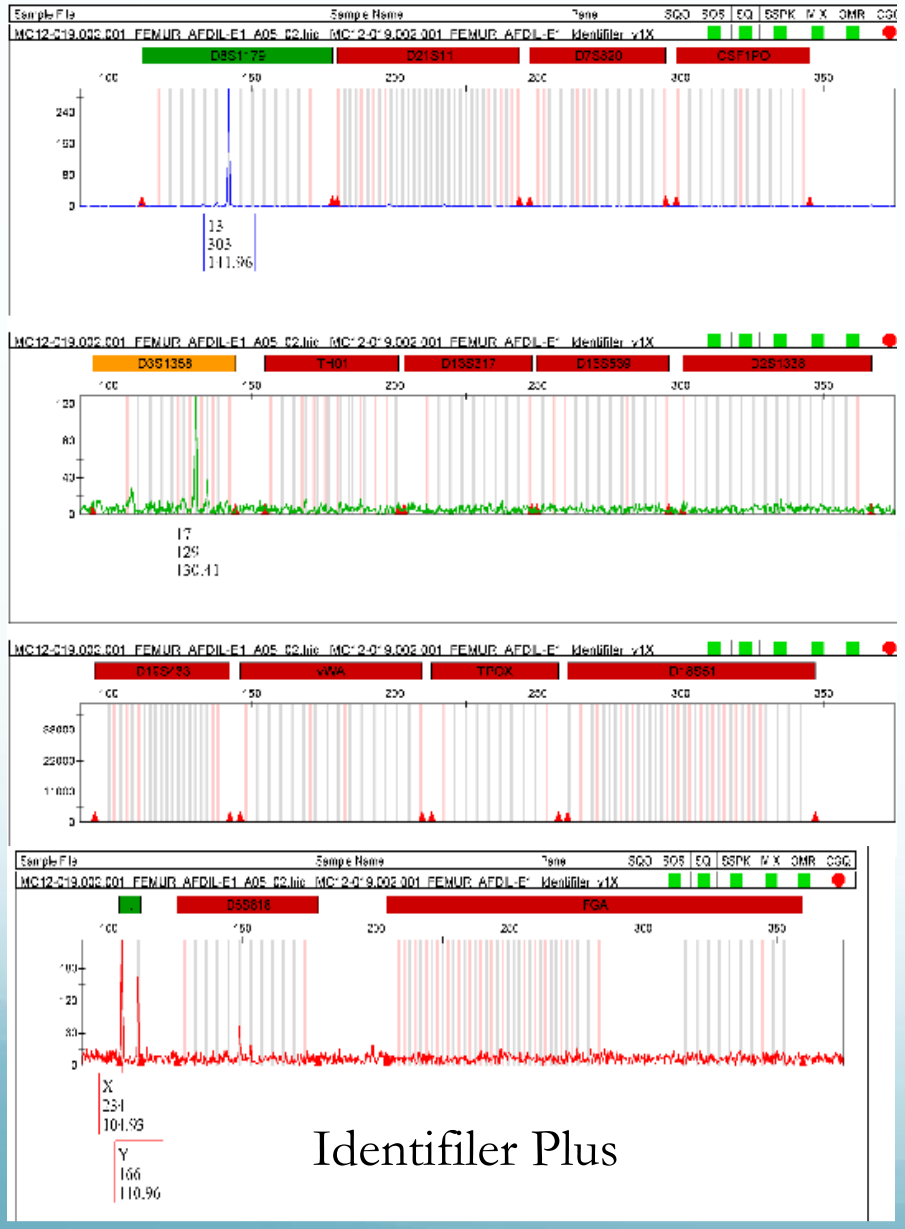
474 RFU

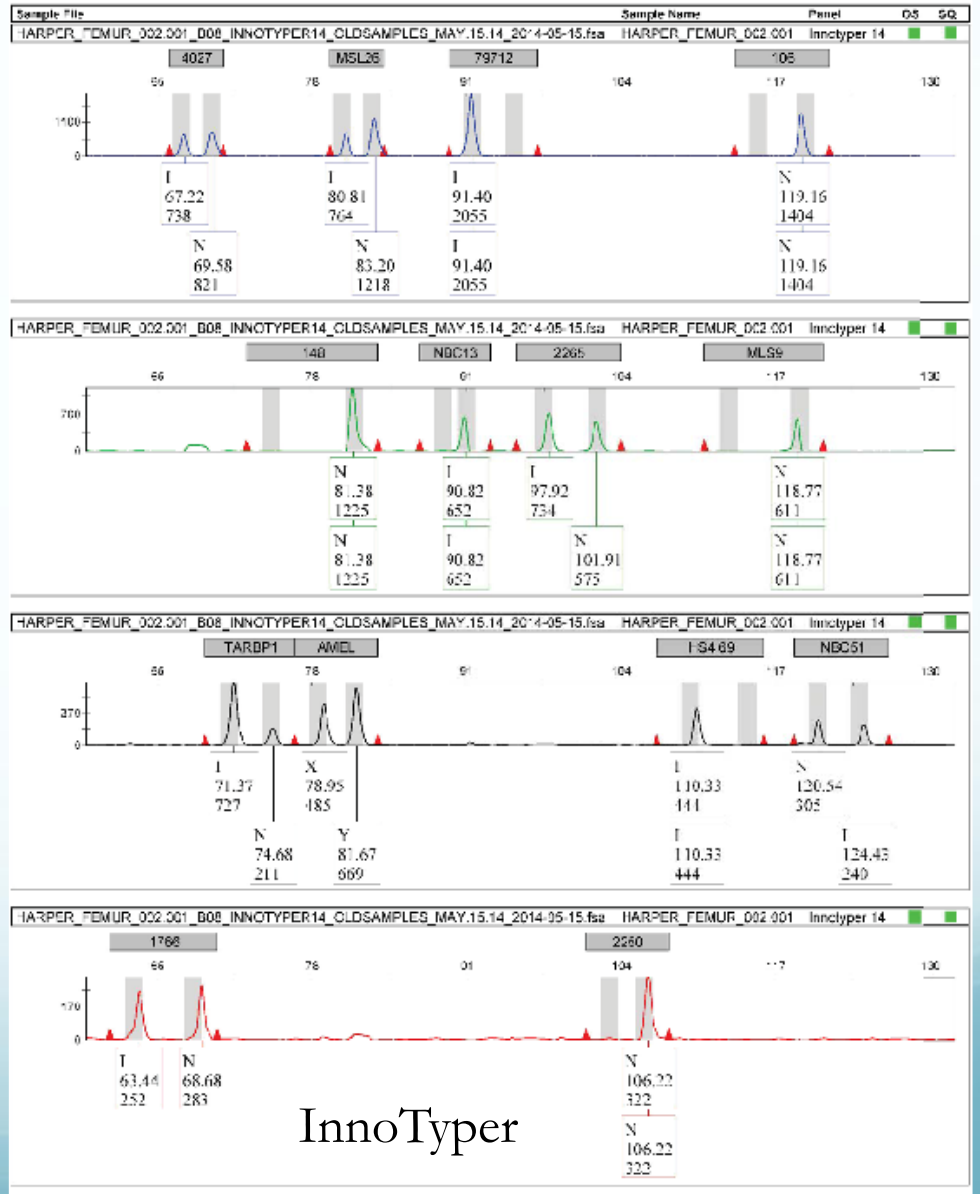
152 RFU

4 cm Hair Shaft – InnoTyper™ Results



InnoTyper and historical remains





NG Typing Kit Summary…

- Stable, well characterized and published markers with a number of appealing genetic attributes, inherited by descent only.
- Ability to analyze degraded nuclear DNA, ideal for use with samples requiring mtDNA analysis.
- High Power of Discrimination: greater than mtDNA.
- Ideal for mass disaster testing of highly compromised samples.
- Can be utilized with existing or RDIS, next-gen platforms
- Can provide information regarding bio-ancestral origin and sex of an unknown sample.
- Like other Bi-Allelic systems, not yet suitable for mixture analysis using standard methods.

Conclusions

- Next-generation (NG) systems are now available to improve sample processing and profile success in a forensic lab
- Quantification & Degradation Assessment kit provides additional information prior to PCR amplification that will significantly reduce downstream re-processing and enable DNA analysts to make decisions informatively
- *Alu* based typing kit provides usable profile data for samples that are highly degraded where other systems fail

Acknowledgements

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Thank You