Development of New Technologies for DNA Analysis of Challenging Forensic Samples

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InnoGenomics
Innovation in Forensic Genetics

Commercial interest

The presentation is for scientific and educational purposes only

No financial interest in the following products:

Amicon DNA Concentrators, Identifiler® Plus, MiniFiler™, Quantifiler®, 7500 Real-Time Instrument, SDS and HID software, GeneMapper, GeneMapper-IDX, PowerPlex® S5, PowerPlex® 6C Fusion, Profiler, Cofiler, Globalfiler®, ForenSeq™ Kit, Quantifiler Trio.

Financial interest in the following products:

InnoQuant™ and InnoTyper™ produced by InnoGenomics Technologies, LLC

The Problem

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

- Poor quality with 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. 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
   - Compatibility of platform

   (i.e. InnoQuant™)

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

   (i.e. InnoTyper™)

InnoQuant™

Quality and Quantity assessment system

- Three target qPCR assay:
  - Autosomal target of 80 bp (>1000 copies/genome)
  - Autosomal target of 207 bp (>1000 copies/genome)
  - Synthetic IPC for detection of inhibition
- Use of this 3-target qPCR 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

InnoQuant™ Primer Design

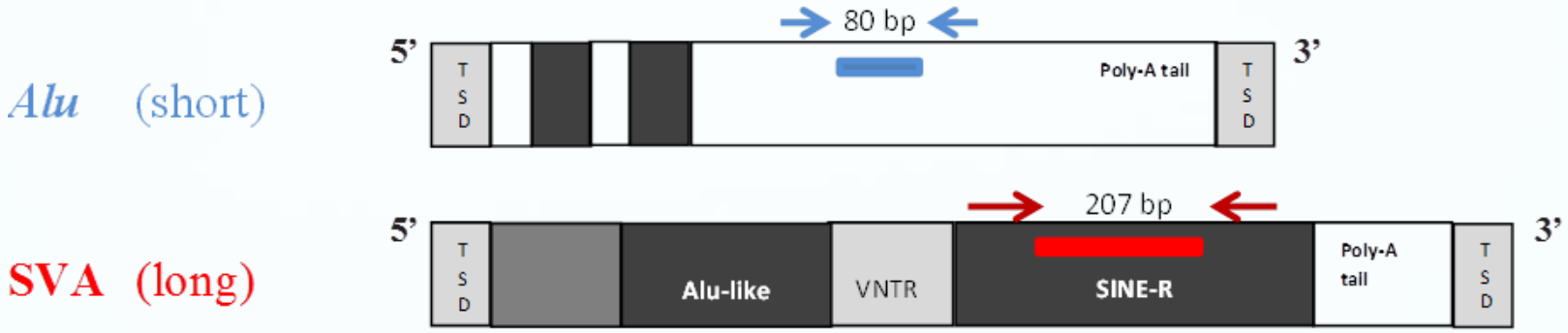

Figure 1. Illustration of *Alu* and SVA (full-length retrotransposons are not drawn to scale). As represented, the REs have a target site duplication (TSD) consisting of identical DNA sequences at the beginning and end.

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Development and validation of InnoQuant™, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements *Alu* and SVA

Gina M. Pineda, Anne H. Montgomery, Robyn Thompson, Brooke Indest, Marion Carroll, Sudhir K. Sinha *

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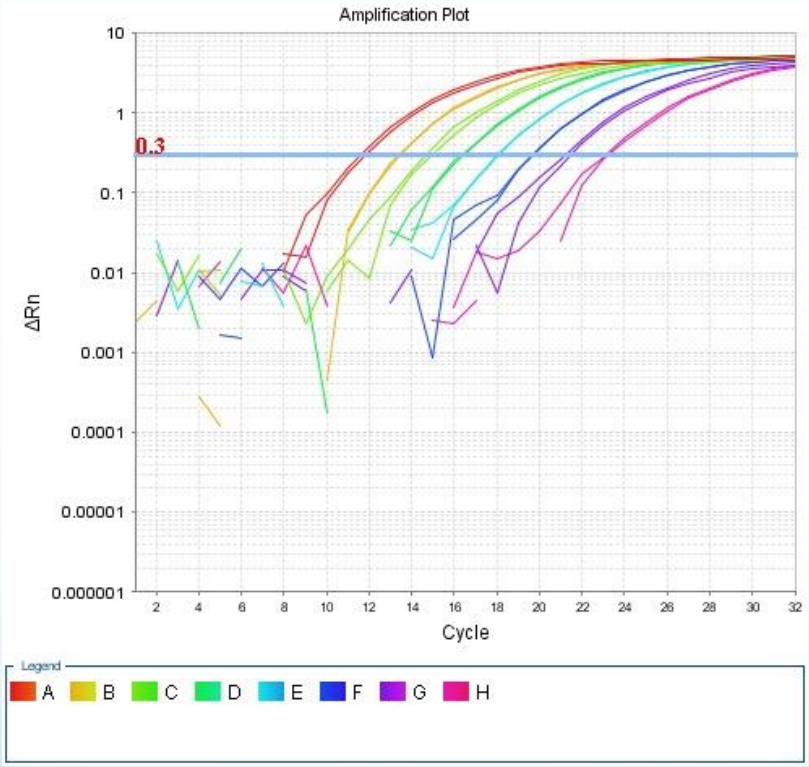
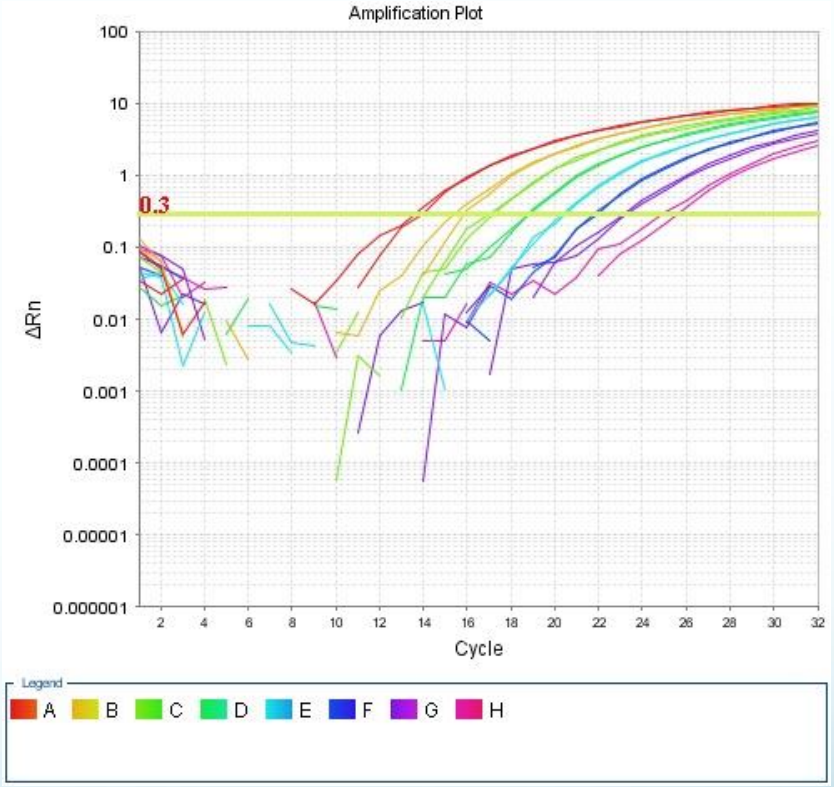
Real time PCR amplification plots

Short Target

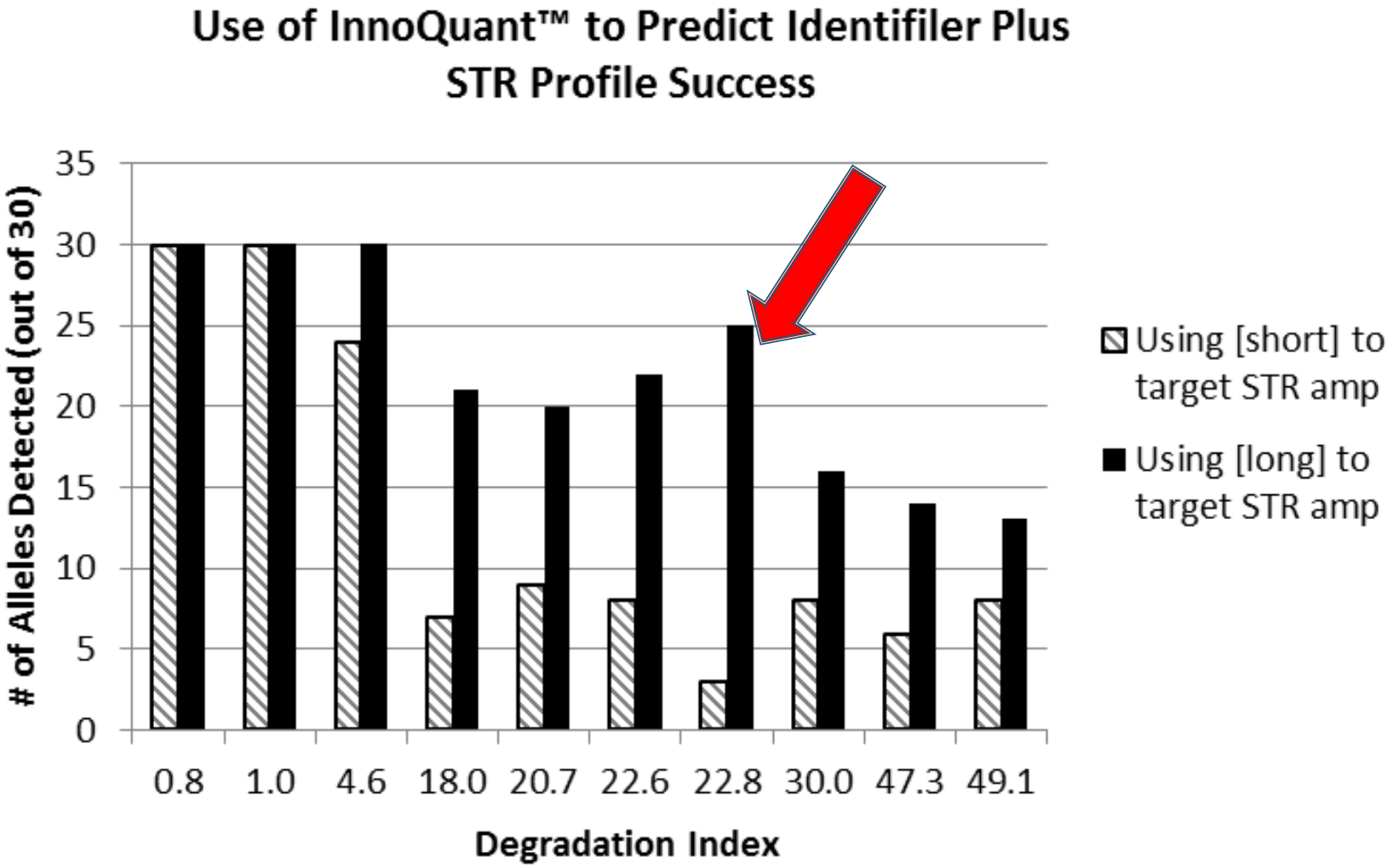


Long Target

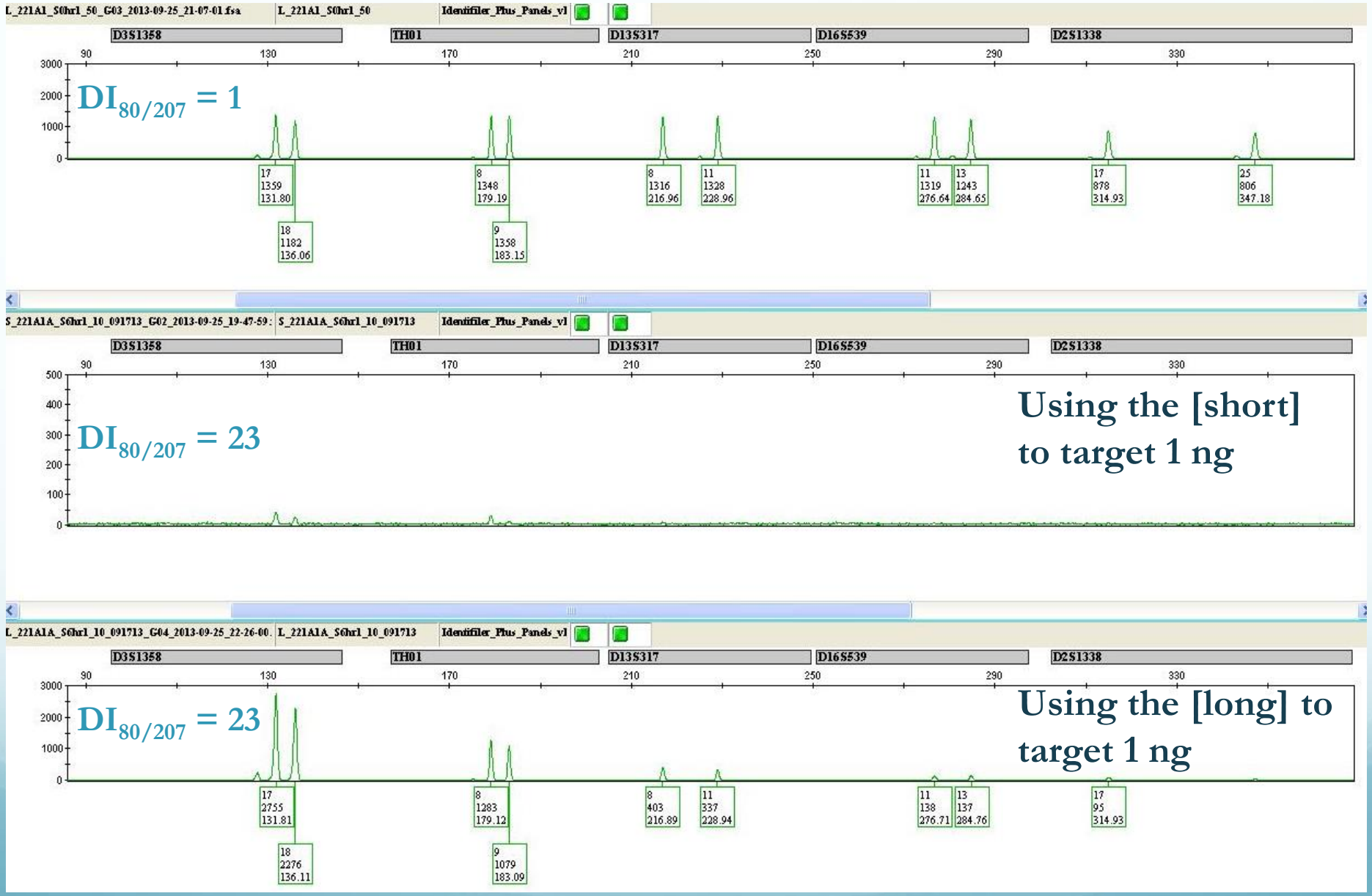


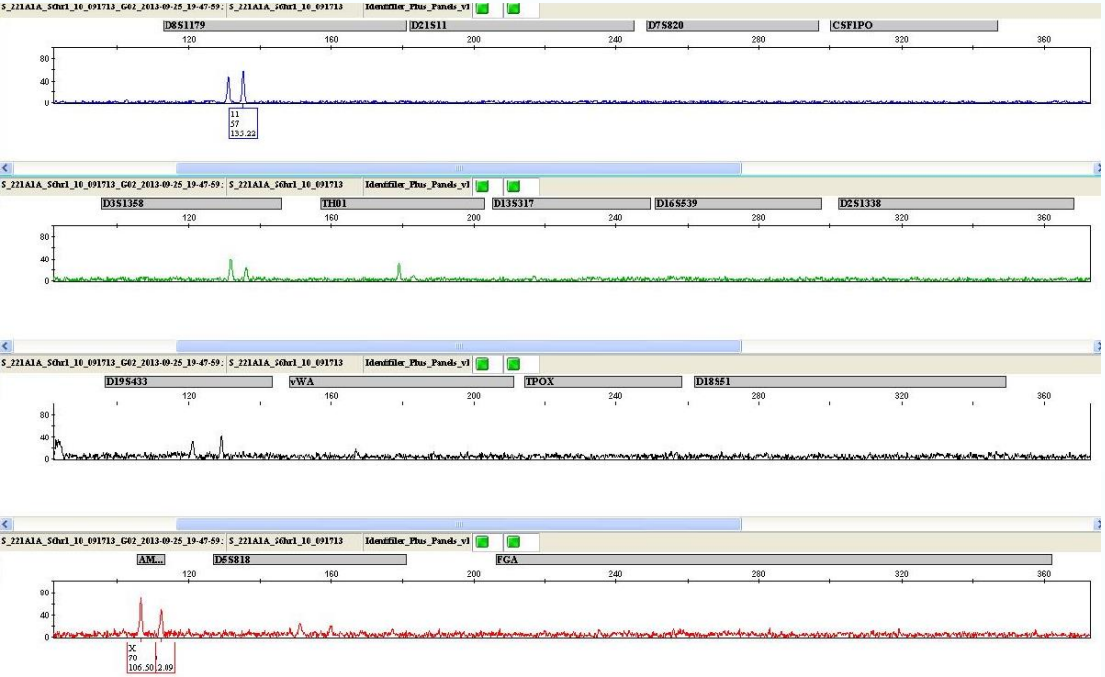
PCR efficiency:	96.689%	98.153%
Slope:	-3.404	-3.367
R^2:	0.998	0.996

Figure 10 from FSI:G paper: Degradation Study



ID Plus Electropherogram: Green



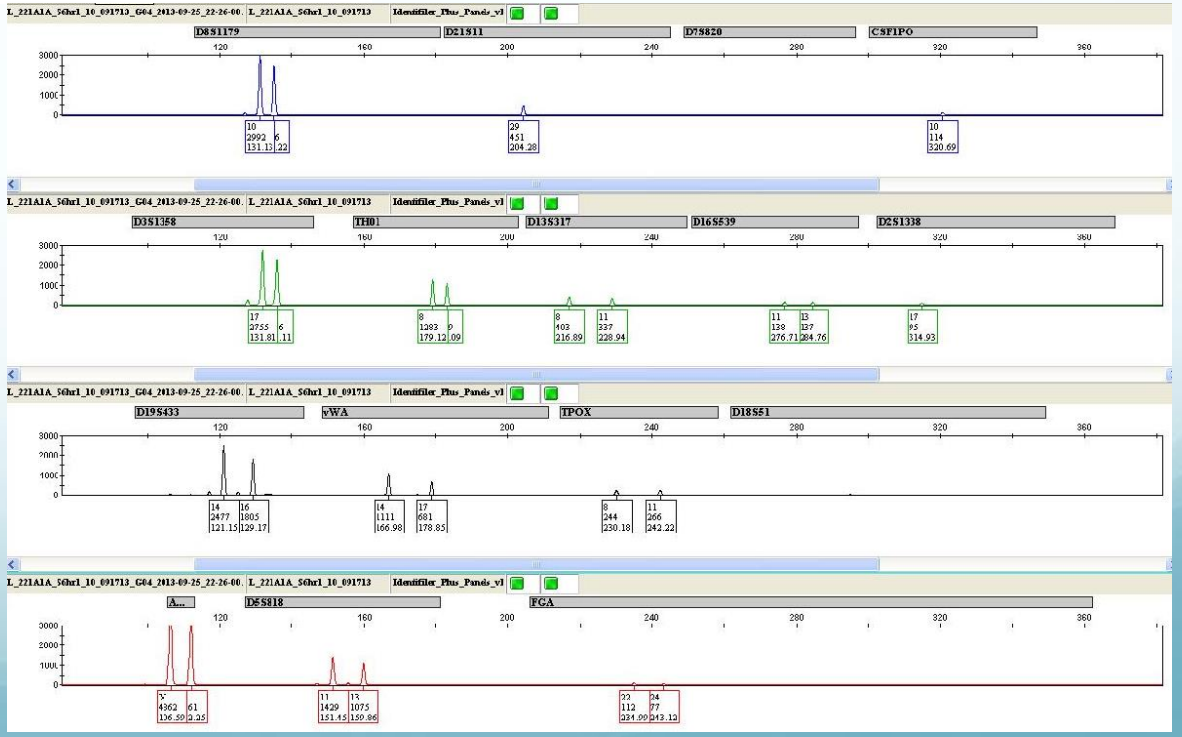


Using the [short] to target IDP amp:

- 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



Degradation of Developed Fingerprints

Data from Reena Roy and Zachary Goecker at Penn State University Forensic Science Program (see Stephanie Plazibat's talk #B110 on Thursday, February 19, 2015 at 2:00 PM)

- Three latent fingerprints were each collected on a glass slide. Slides were then aged 1 day before development.
  - Development techniques include black powder dusting, cyanoacrylate fuming, and chalcogenide CTF
  - Fingerprints were aged 1-6 weeks from the point of development to the point of extraction. Aging was done at ambient conditions.
  - Fingerprint extraction was done using an LCN protocol which involves pK digest and Amicon concentration.

Degradation of Developed Fingerprints

Quantitation values (pg/uL) from short amplicon using InnoQuant™

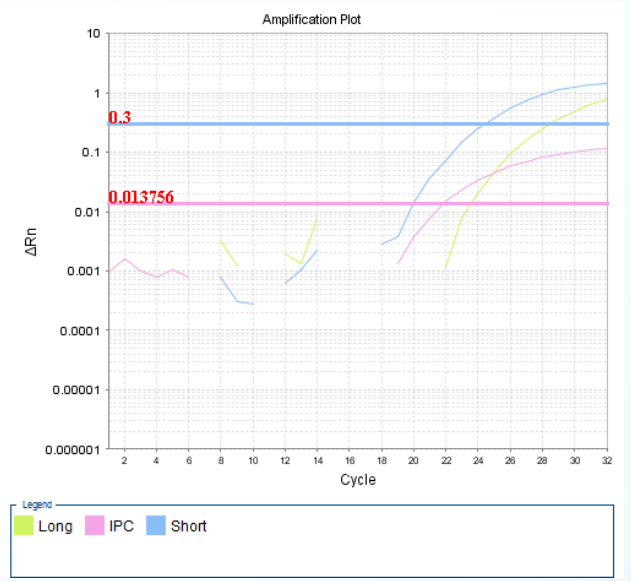
Donor 1	CTF	Cyanoacrylate	Powder	Not enhanced
1 Week	23.3	55.9	10.6	34.6
2 Weeks	6.0	10.6	6.4	21.3
3 Weeks	15.6	12.3	2.1	9.5
4 Weeks	16.0	Undetermined	11.6	Undetermined
5 Weeks	9.5	12.8	9.0	776.0
6 Weeks	7.3	23.5	11.4	17.0

Average quantity of DNA across all developed prints:

14 pg/µl

Degradation Indices ([short]/[long]) using InnoQuant™

Donor 1	CTF	Cyanoacrylate	Powder	Not enhanced
1 Week	4.75	3.23	3.87	3.12
2 Weeks	5.85	4.98	3.95	3.31
3 Weeks	4.33	4.92	4.39	5.11
4 Weeks	4.75	Undetermined	4.57	Undetermined
5 Weeks	5.72	4.04	5.31	2.56
6 Weeks	5.72	3.36	4.91	4.31



Fingerprints developed with Columnar-thin-film aged 6 weeks at ambient conditions

InnoQuant™ with Casework Samples

- **Data provided by Dr. Aaron LeFebvre at Cellmark**
- 216 property crime samples tested with InnoQuant™
- Previously tested with Quantifiler® Human and Identifiler Plus (half reaction with a target input of 500 pg)
- Most samples that did not produce a result with Quantifiler® Human did produce a result with at least the InnoQuant™ short target

Property Crime Samples and InnoQuant™

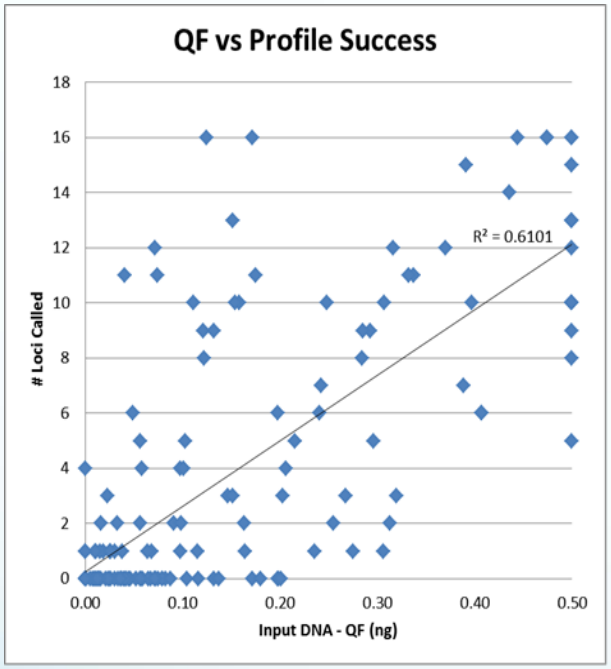
System	Samples w/ no Quant Value	Percentage
InnoQuant (Short target)	5	2.3%
InnoQuant (Long target)	45	20.8%
Quantifiler Human	66	30.6%

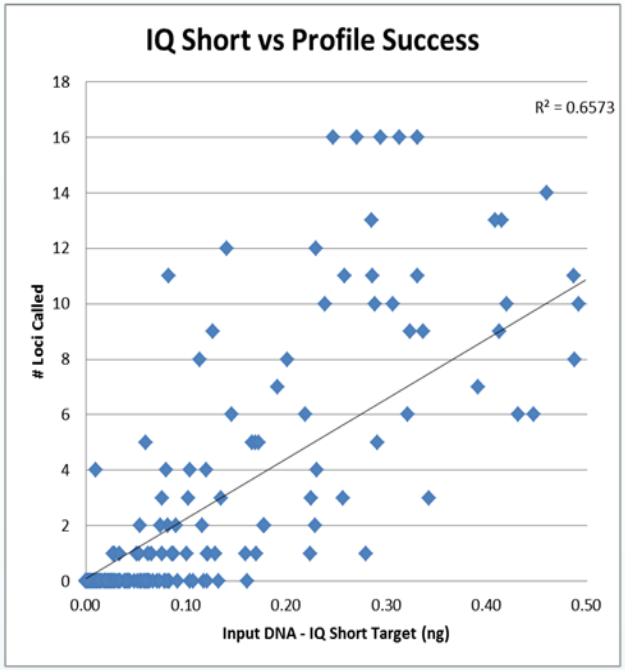
Indicates higher sensitivity of InnoQuant™

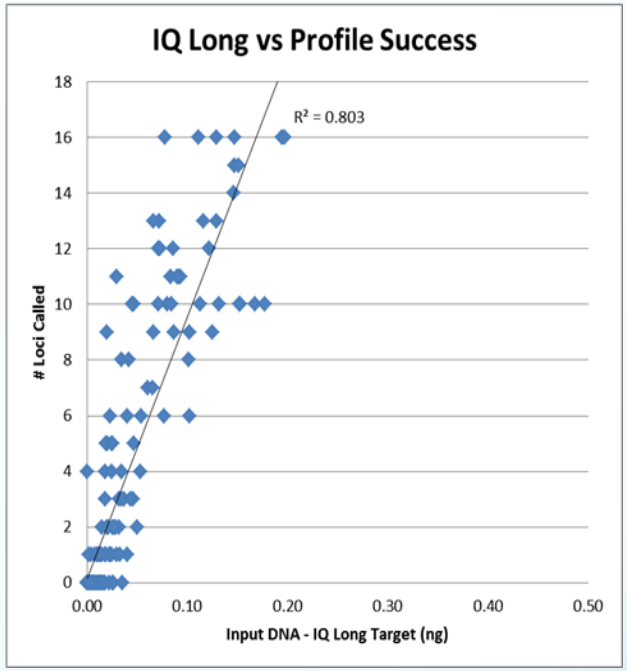
Most samples (75%) had a $DI_{80/207}$ between 3-10, which indicates that a large percentage of forensic samples have some moderate degradation, which may cause issues with targeting and result in unnecessary rework

DI	# Samples	Percentage
<3	28	16.4%
3-5	68	39.8%
5-10	61	35.7%
10-15	7	4.1%
15-20	3	1.8%
>20	4	2.3%

Use of InnoQuant™ to Predict STR Success







Based on sample available and IQ long target, assessment can be made whether an IDP profile is likely and whether or not other options are more suitable for the sample (such as sample concentration, MiniFiler, mtDNA or InnoTyper™)

Use of InnoQuant™ to Improve 1st Pass Success Rates

DI	Number of Samples	Percentage	# of Samples with QF > 0.1 ng/uL	# w/Full Profile on 1st Pass	% Full Profiles on 1st Pass
<3	28	16.4%	8	8	100%
3-5	68	39.8%	9	3	33%
5-10	61	35.7%	7	0	0%
10-15	7	4.1%	0	0	0%
15-20	3	1.8%	0	0	0%
>20	4	2.3%	0	0	0%

If samples were properly targeted with IQ, the first pass success would improve.

Summary 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 target DNA to use
- Compatible with current platforms (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 samples fall 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 next generation systems with small amplicon sizes

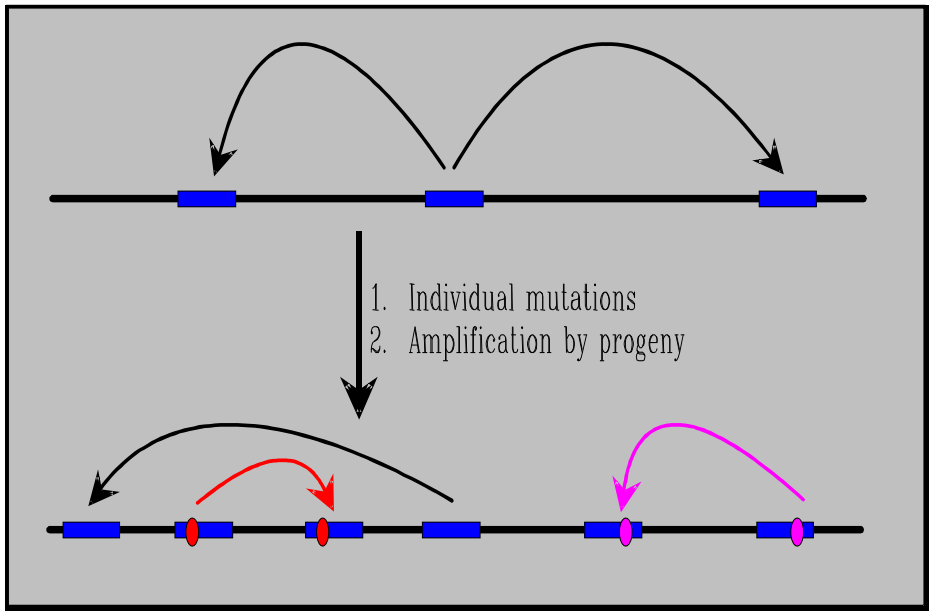
InnoTyper™

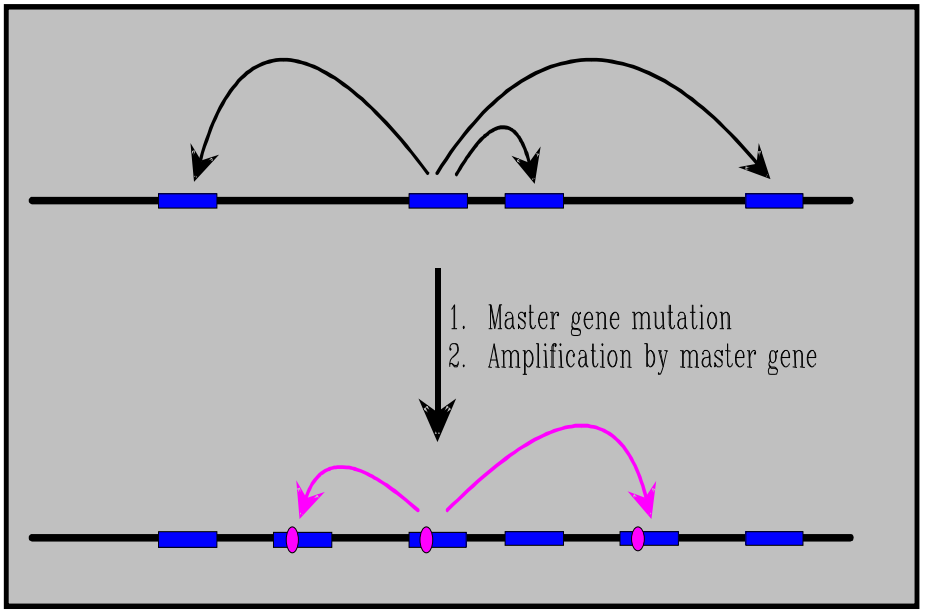
A mobile element based Small Amplicon DNA Typing System

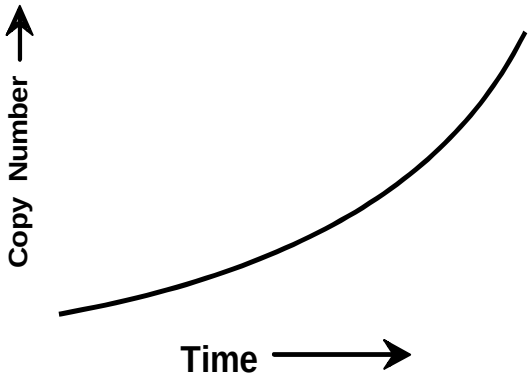
Structure of Alu Element

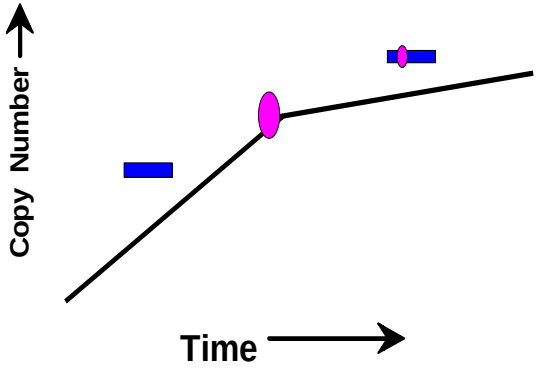
- 300 bp long
- RNA polymerase III transcribed
- 3' oligo dA-rich tail
- 500,000 copies in human genome
- most amplification 40 mya
- similar copy # in human & higher primates
- dimer-like structure
- poorly transcribed

Transposon vs. Master Gene Models

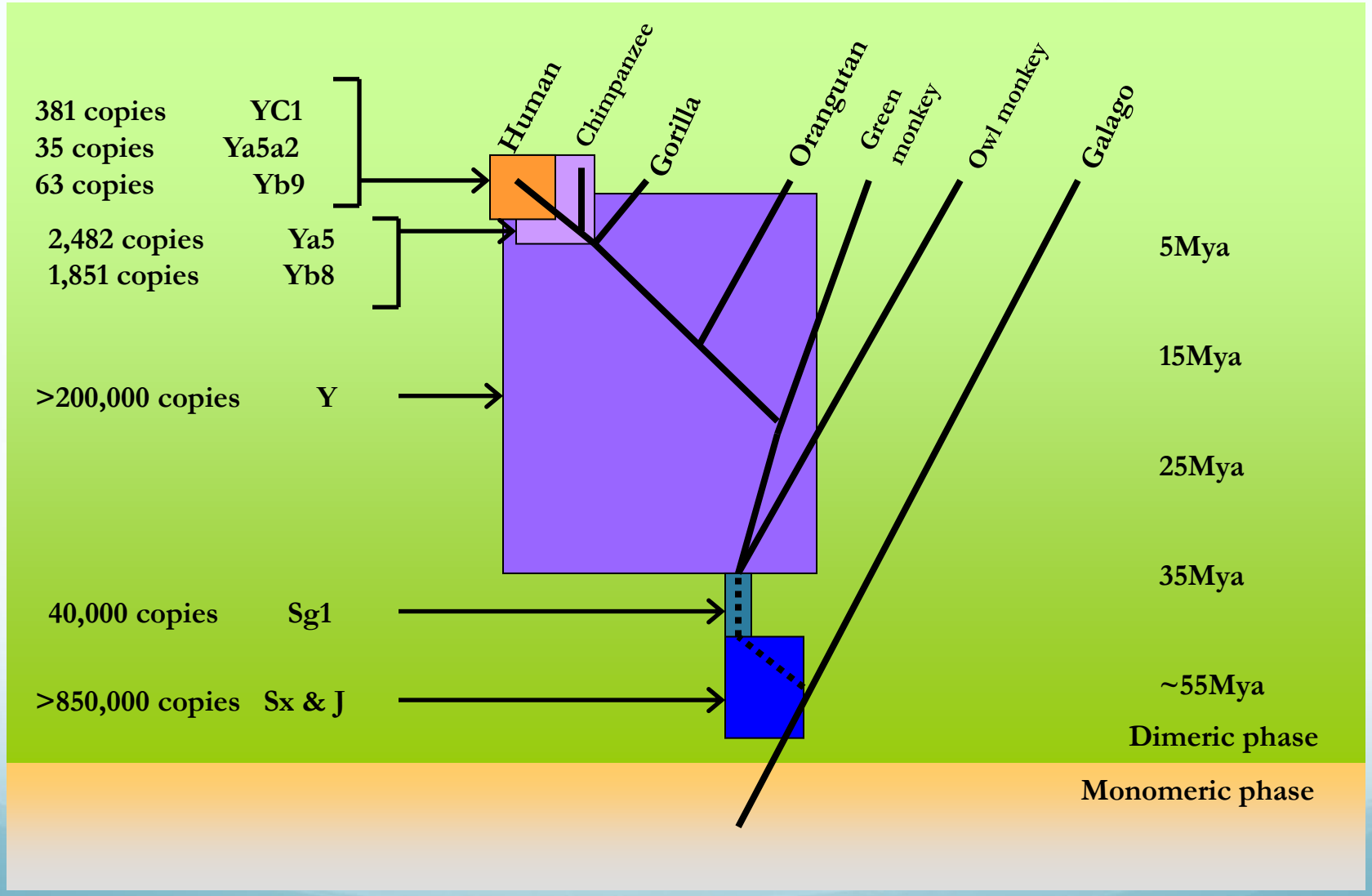








Mobile Elements

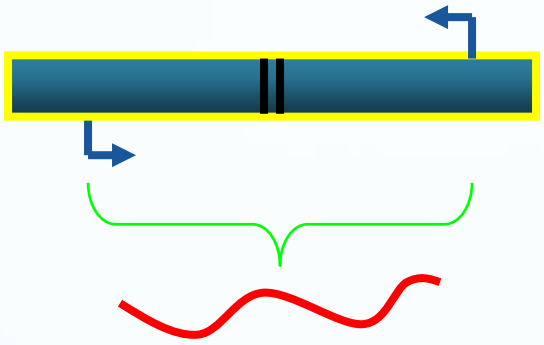


Batzer and Deininger (2002) *Nature Genetics*

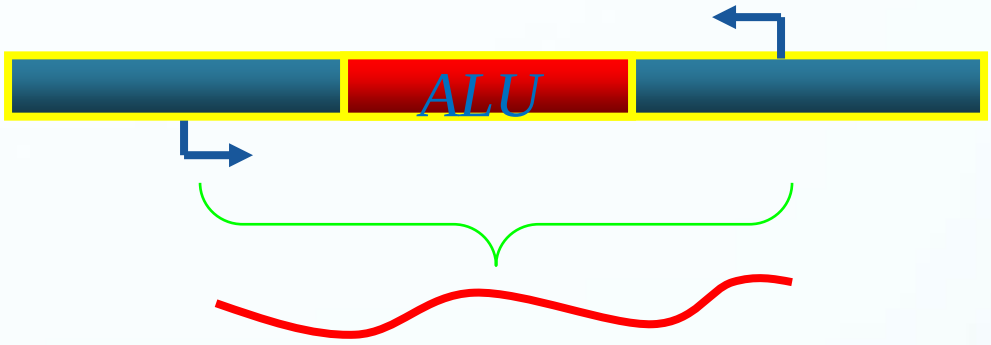
Properties of Mobile Element Insertions

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 (unlike STRs or SNPs)

Old Alu Multiplex Design



Amplification product
~100bp (N)

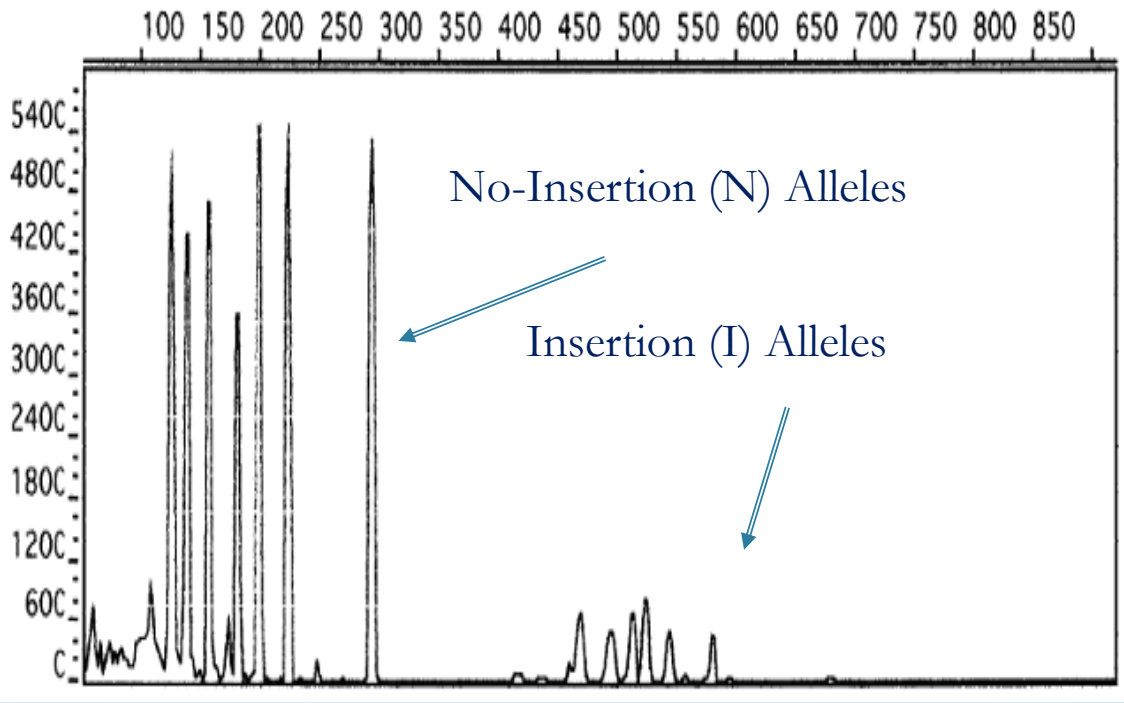


Amplification product
~400bp (I)

1. **Homozygous Insertion = I, I**
2. **Heterozygous = I, N or N, I**
3. **Homozygous No Insertion = N, N**

Caused preferential amplification of empty sites due to 300 bp allele size difference between I and N (allelic drop-out)

Alu multiplex with original primer design

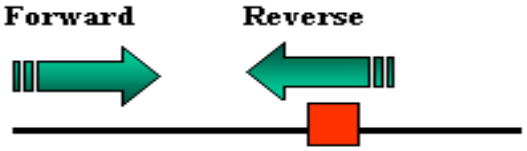


New Multiplex Design

A. Filled Site Reaction

Ccagttgttgaggggaacaaactaaata**agaagagtgaatgcacatttatga**aagtgtaaatgaacgattcTgttatgaacacaaacatg
accagggtgccgagccttatcatt**AAGAAACTG**GGCCGGGC**GCGGTGGCTCACGCCTGTAATCC**
CAGCACTTTGGGAGGCCGAGGCGGGCGGATCACGAGGTCAGGAGATCGAGACCATC
CCGGCTAAAACGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCCGGGCG
TAGTGGCGGGCGCCTGTAGTCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATGGCG
TGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATCCCGCCACTGCACTGTCCAG
CCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAAATAAATAAATAAATAAATAAA
AGAAACTGaattcatgactcccagtctgggggaacagaaaacattactgagctggagcacattggcc

B. Empty Site Reaction



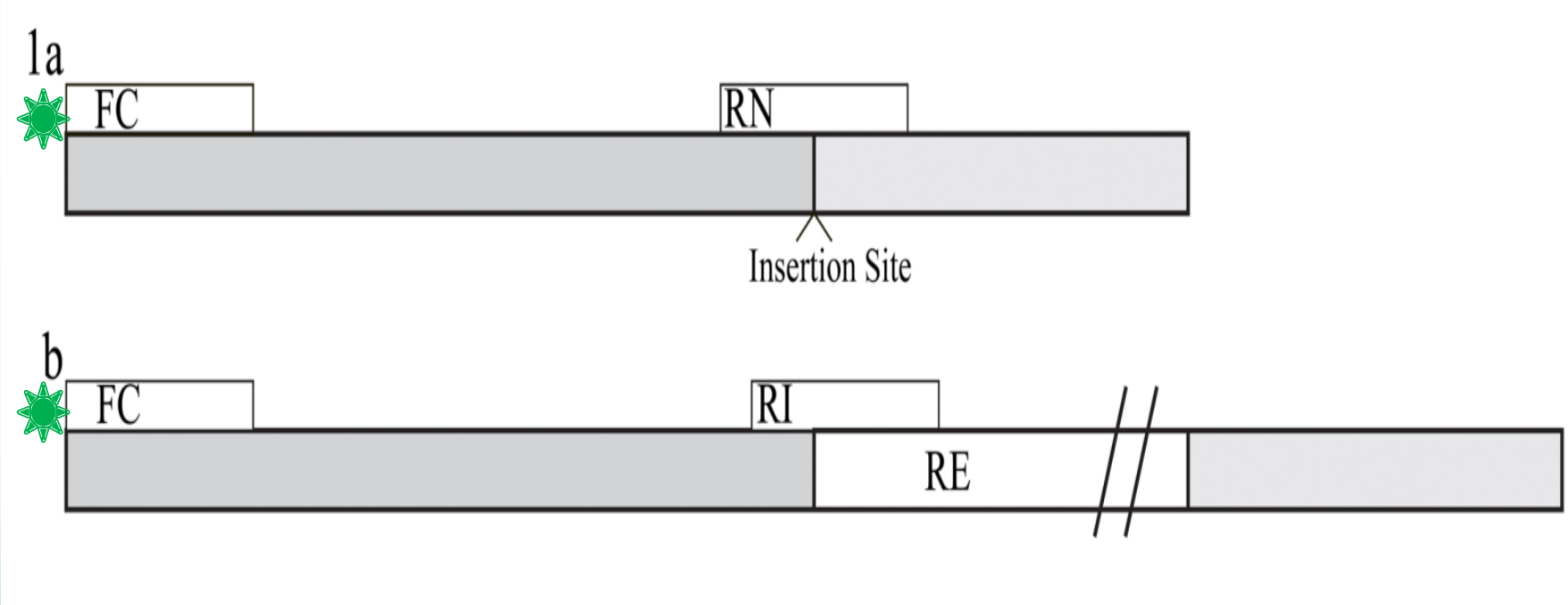
Ccagttgttgaggggaacaaactaaata**agaagagtgaatgcacatttatga**aagtgtaaatgaacgattctgttatgaacacaaacatg
accagggtgccgagccttatcatt**AAGAAACTG**aattcatgactccc**agtctgggggaacagaaaacattactgagctggagca**
cattggcccattcagttctgaaatgcatgtccaacaggtaggtggagccctgagcaagaattgctactgtgttgaagtcacagagccag

Carter and Sinha, US Patent Application #60/902,850

Method for Genetic Detection using Interspersed Genetic Elements

Novel mini-primer Design

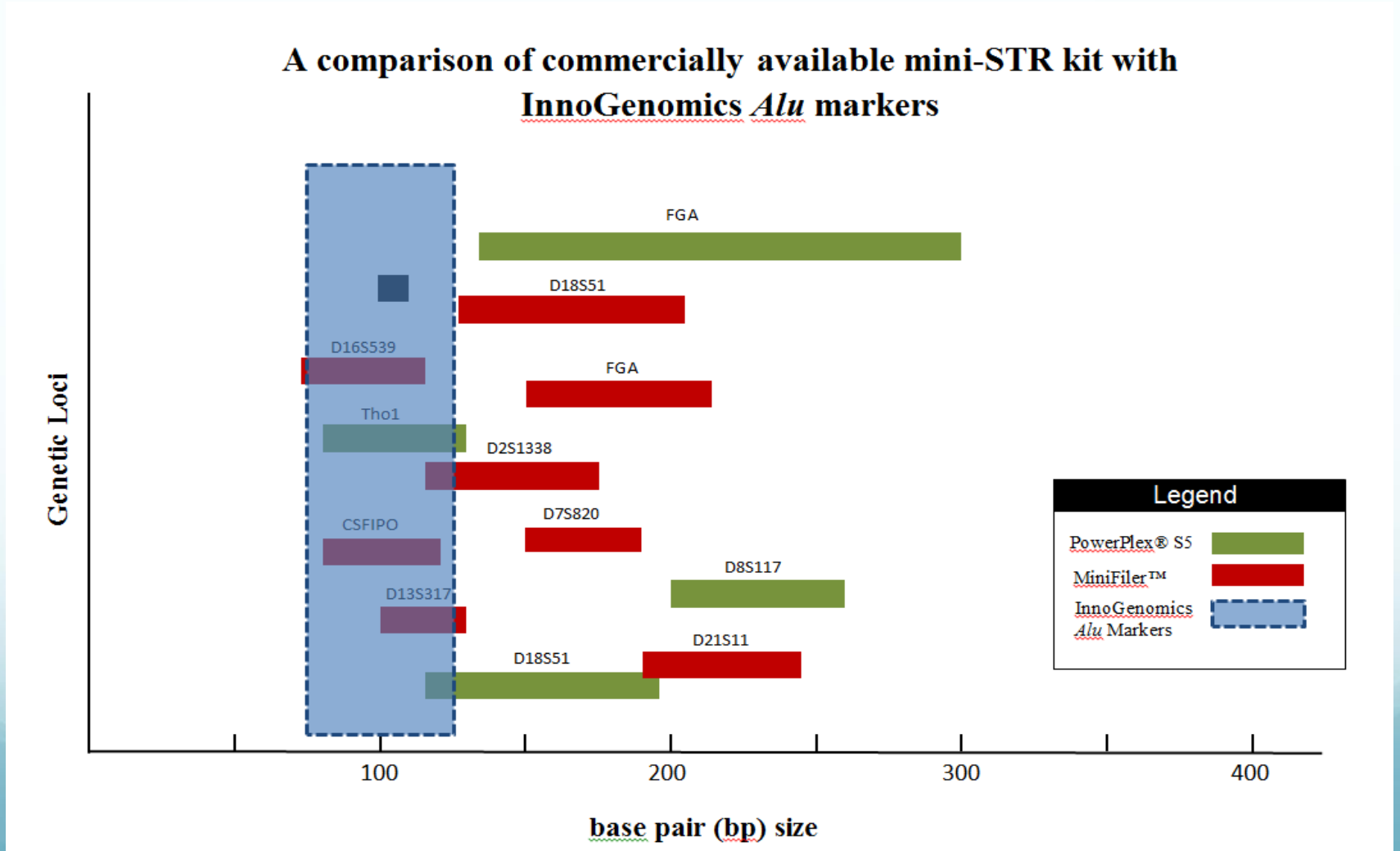
Labeled (common) forward primer and unlabeled reverse



InnoTyper-21

- 20 markers + Amelogenin multiplex
- System amplifies *Alu* sequences less than 125 bp
- This system provides:
  - High sensitivity
  - High tolerance for degraded samples
  - High discrimination power (~1 in 100 million)

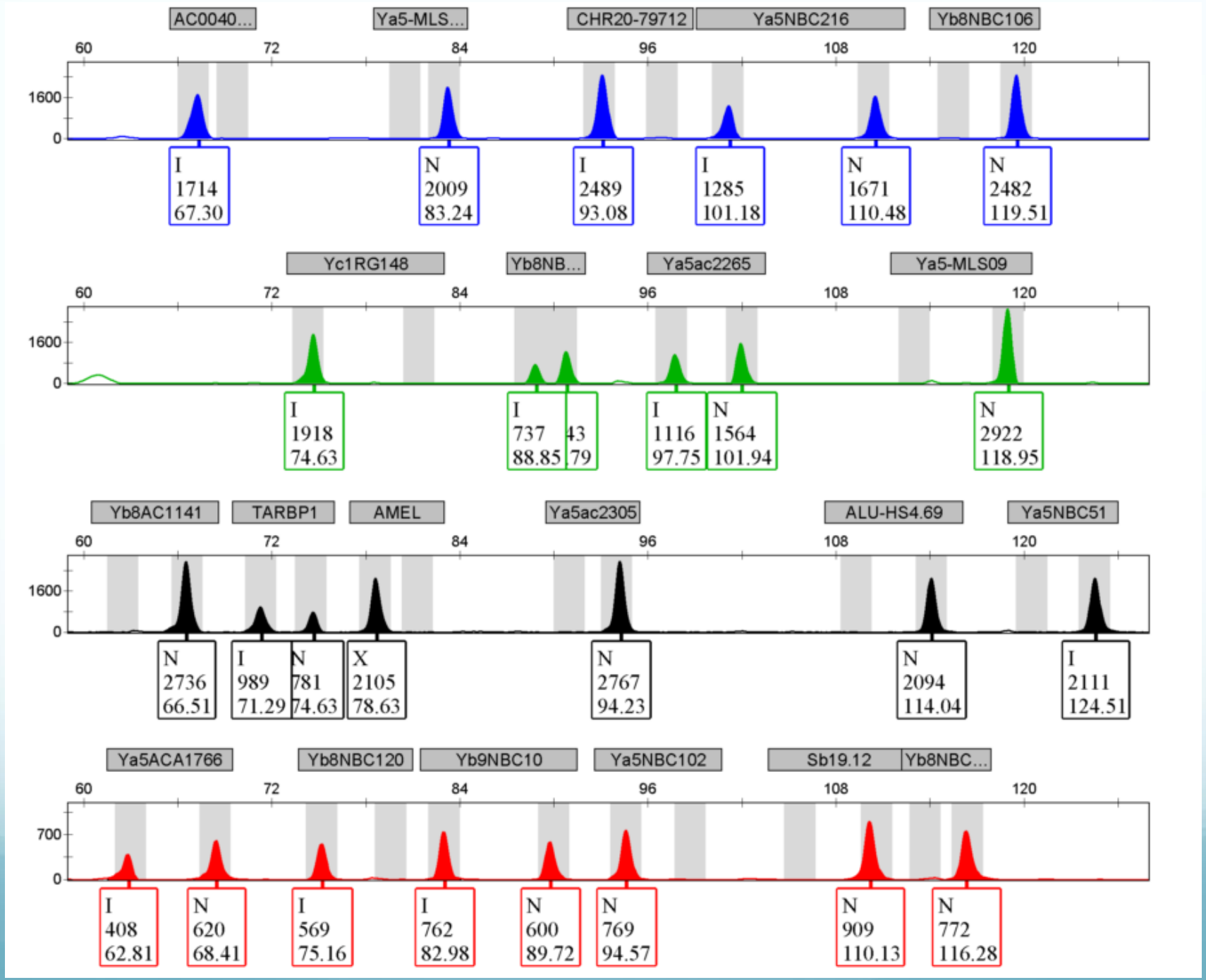
BP Size Comparison of mini-STR kits with InnoGenomics markers



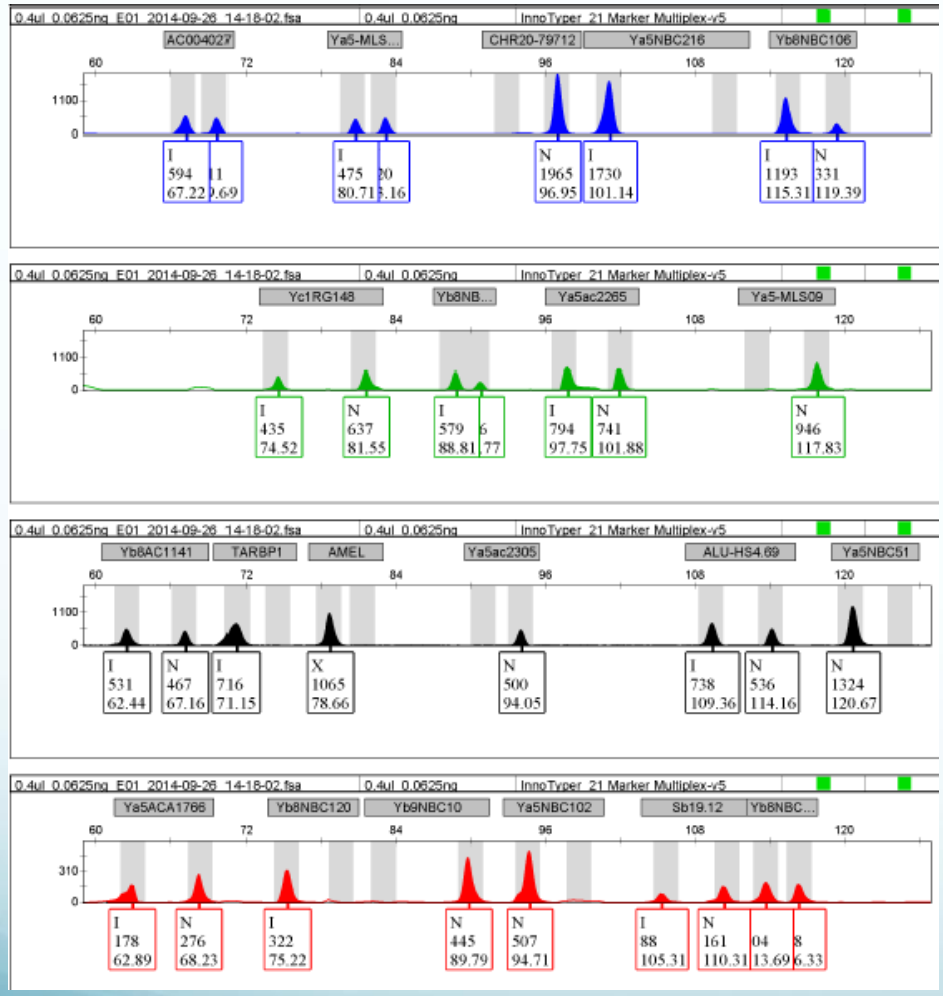
InnoTyper™ 21 Database Samples

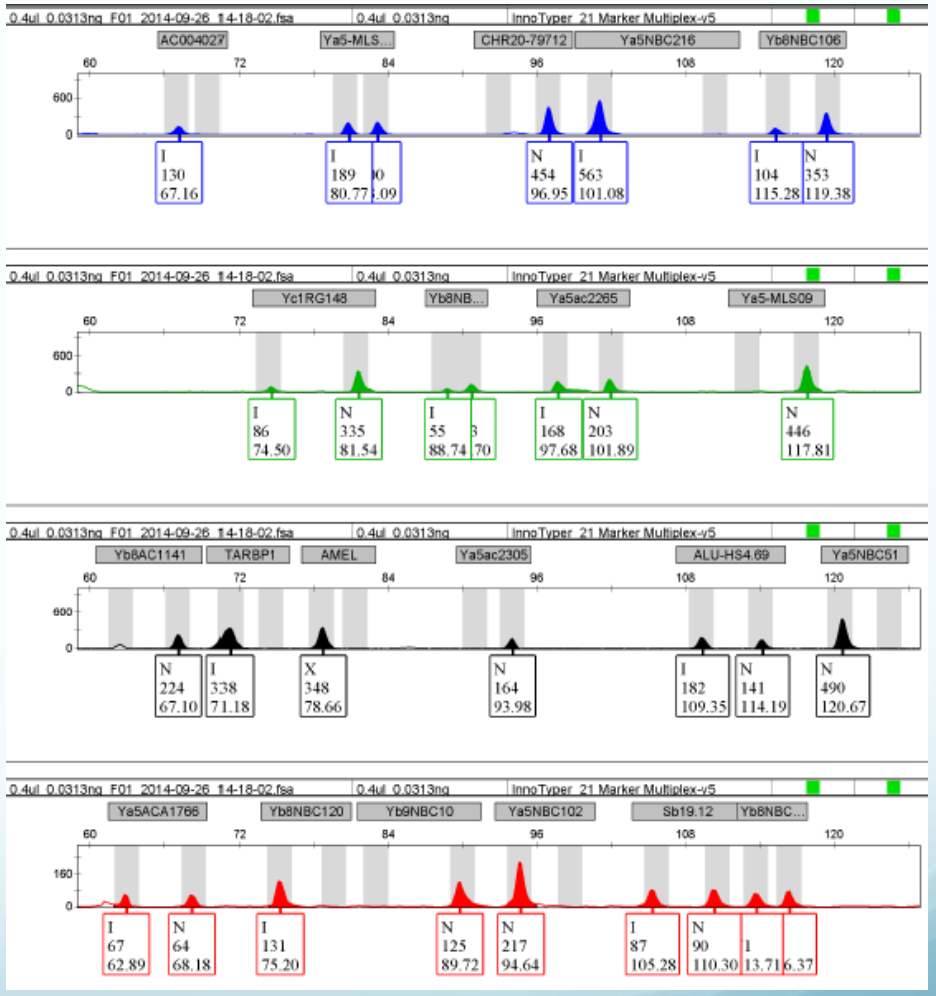
- Four US populations:
  - In-house: Caucasian (206), African American(201)
  - UNT: SW Hispanic (45), Asian American (44)
- Additional Caucasian and African American:

  100 Anonymous Paternity Trios (~200 M and AF samples) with STR data and known Exclusion and Inclusion results.
- Some Cauc & AA were environmentally degraded samples: 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

Hair Shaft Study

Hair samples were purified and provided by Dr. Mark Wilson's Lab

Sample Preparation:

- Three hair samples and reference buccal swabs were collected from ten individuals
- A follicular tag (0.5cm) was removed with UV irradiated scissors. 2 cm fragment of hair was then cut from this end
- Hairs were cleaned via a series of washes
- DNA was extracted from this 2 cm hair shaft
- DNA extract was vacuum concentrated

Hair Shaft InnoQuant™ Results

Donor ID	Large	Small	DI
1-1	0.00000	0.00011	
1-2	0.00000	0.000115	
1-3	0.00000	0.000153	
2-1	0.002251	0.016421	7
2-2	0.000137	0.00902	66
2-3	0.00000	0.00000	
3-1	0.00000	0.000243	
3-2	0.00000	0.00041	
3-3	0.00000	0.000174	
4-1	0.002486	0.040278	16
4-2	0.001315	0.028299	22
4-3	0.000947	0.016411	17
5-1	0.00000	0.000424	
5-2	0.00000	0.000908	
5-3	0.00000	0.000628	
6-1	0.00000	0.000214	
6-2	0.00000	0.000156	
6-3	0.00000	0.000209	
7-1	0.00000	8.98E-05	
7-2	0.00000	0.00016	
7-3	0.00000	0.00026	
8-1	0.000658	0.012372	19
8-2	0.002011	0.010771	5
8-3	0.00087	0.01506	17
9-1	0.000328	0.017123	52
9-2	0.00000	0.011822	
9-3	0.00000	0.007381	
10-1	0.00016	0.016266	105
10-2	0.000327	0.020693	63
10-3	0.000196	0.02242	114

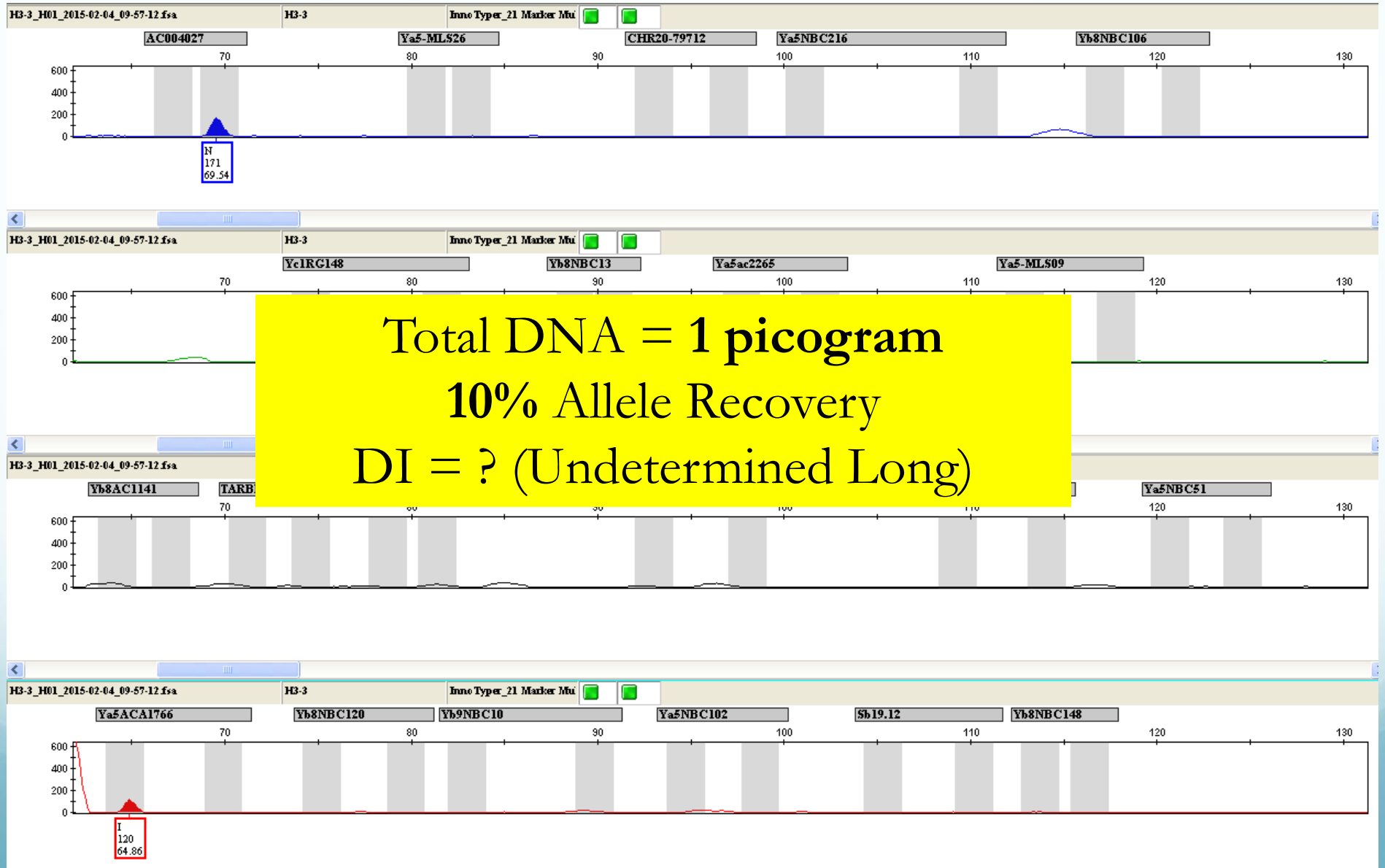
	IQ Long pg/uL	**IQ Short** pg/uL	**Degradation Index**
Average	0.4	8.3	42
Minimum	0	0	5
Maximum	2.5	40.3	114

- Very low quantitation values
- Degradation is present
- Amplified with InnoTyper™ 21
  - Total of 6.2 uL of DNA extract added to amplification

Quantitation comparison for hair shafts with usable InnoTyper™ profiles

	Quantifiler Trio: Genomic Quant (ng/ul)			InnoQuant™: Genomic Quant (ng/ul)		
Donor ID	**Large**	**Small**	**DI**	**Large**	**Small**	**DI**
2-1	0.0004	0.00135	3		0.00326	
2-2		0.001			0.00285	
2-3	0.00025	0.0039	16	0.00021	0.00691	33
4-1	0.00215	0.00935	4	0.00085	0.01617	19
4-2	0.00055	0.00505	9	0.00040	0.01010	25
4-3	0.00125	0.0031	2	0.00034	0.00570	17
8-1	0.00035	0.0018	5		0.003037	
8-2	0.00145	0.00195	1	0.000579	0.002868	5
8-3	0.00055	0.00205	4	0.000244	0.004523	19
9-1	0.0001	0.0027	27		0.005372	
9-2		0.00195			0.003373	
9-3		0.001			0.002134	
10-1	0.0001	0.0016	16		0.004292	
10-2	0.0002	0.00185	9		0.00546	
10-3		0.0016			0.005166	
Average (pg)	**0.7**	**2.7**	**9**	**0.4**	**5.4**	**20**

2 cm Hair Shaft – InnoTyper™ 21 results

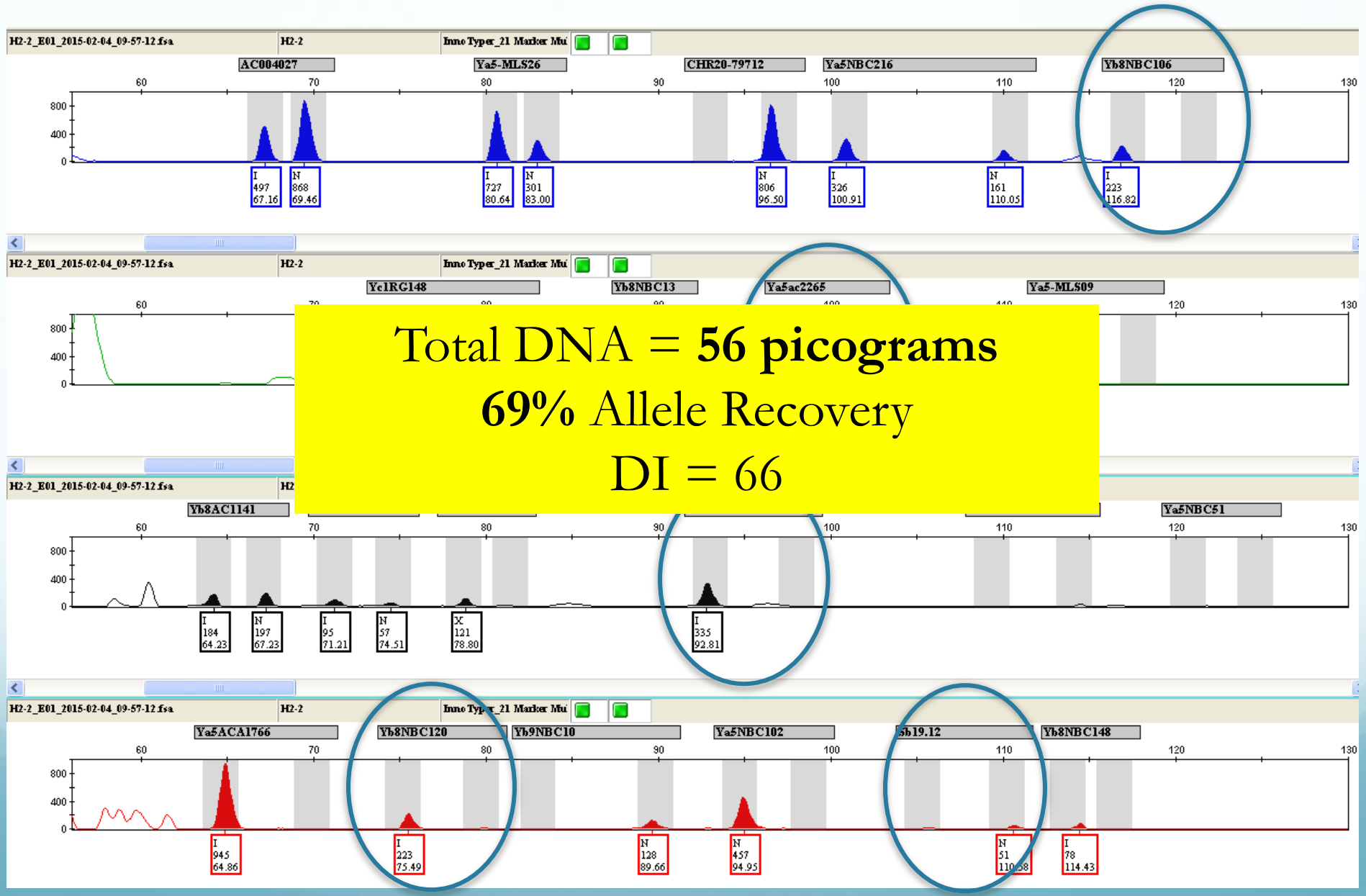


Total DNA = **1 picogram**

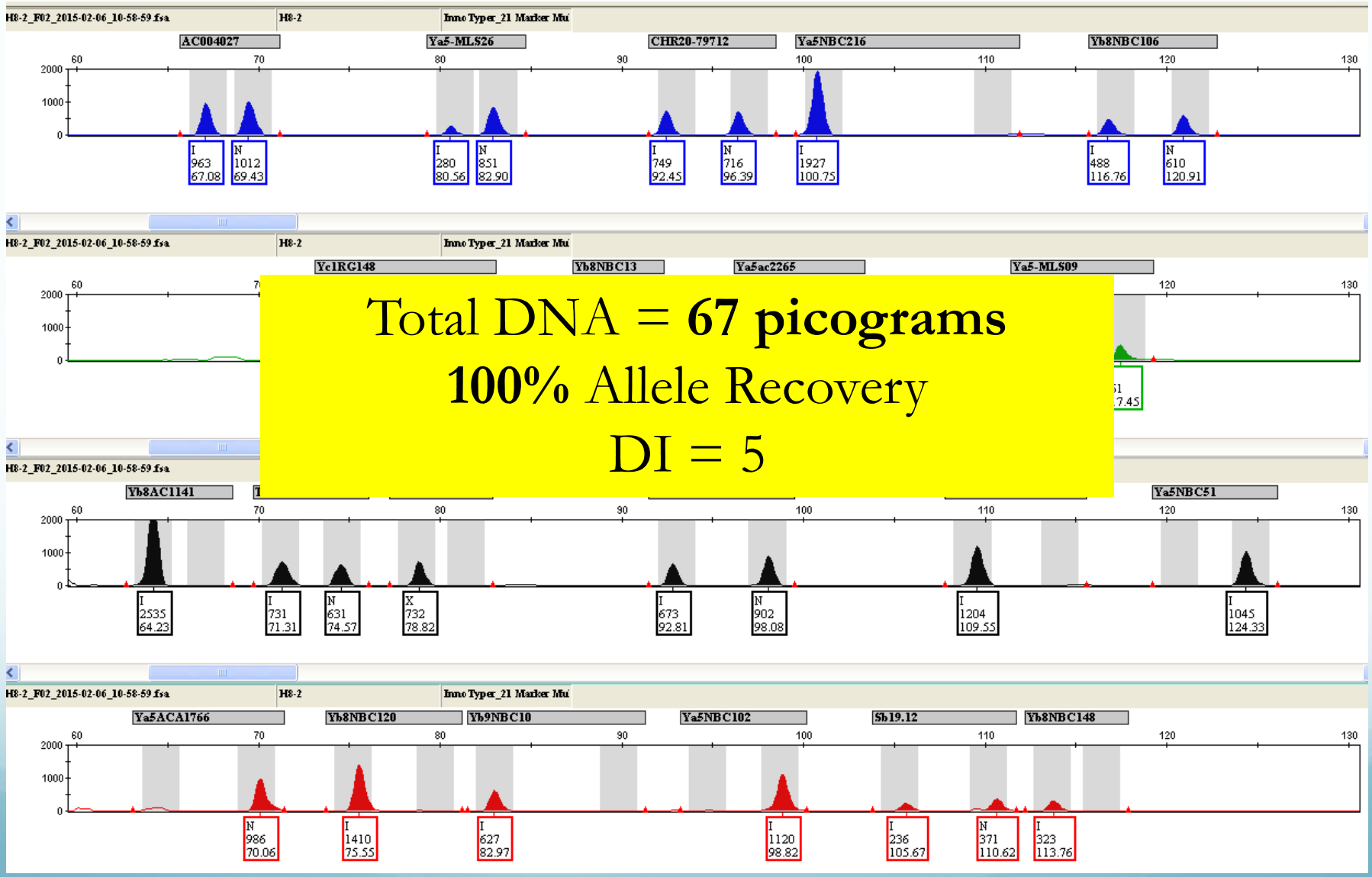
10% Allele Recovery

DI = ? (Undetermined Long)

2 cm Hair Shaft – InnoTyper™ 21 results



2 cm Hair Shaft – InnoTyper™ 21 results

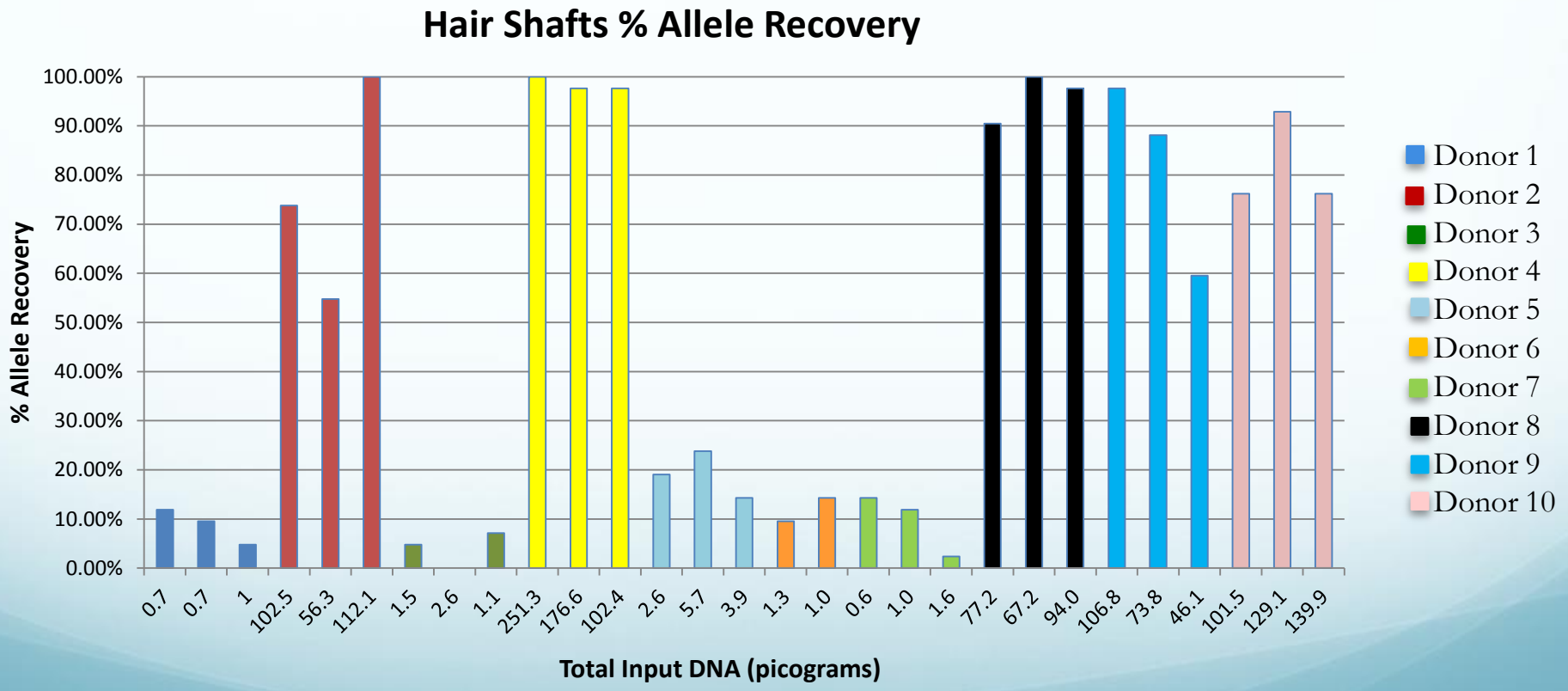


Hair Shaft IT21 Genotype Results

Donor ID	DI	Input DNA (pg)	% recovery	Donor ID	DI	Input DNA (pg)	% recovery
1-1	N/A	0.7	12%	6-1	N/A	1.3	10%
1-2	N/A	0.7	10%	6-2	N/A	1.0	14%
1-3	N/A	1.0	5%	6-3	N/A	1.3	N/A
2-1	7	102.5	74%	7-1	N/A	0.6	14%
2-2	66	56.3	55%	7-2	N/A	1.6	12%
2-3	28	112.1	100%	7-3	N/A	1.6	2%
3-1	N/A	1.5	5%	8-1	19	77.2	90%
3-2	N/A	2.6	0%	8-2	5	67.2	100%
3-3	N/A	1.1	7%	8-3	17	94.0	98%
4-1	16	251.3	100%	9-1	52	106.8	98%
4-2	22	176.6	98%	9-2	N/A	73.8	88%
4-3	17	102.4	98%	9-3	N/A	46.1	60%
5-1	N/A	2.6	19%	10-1	105	101.5	76%
5-2	N/A	5.7	24%	10-2	63	129.1	93%
5-3	N/A	3.9	14%	10-3	114	139.9	76%
Over 50% Genotype Recovery				Less than 50% Genotype Recovery			

Hair Shaft IT21 Genotype Results

- Ten donors (30 hair shafts) were amplified with IT21
- 50% of rootless hair shafts produced usable, interpretable profiles



Hair Shaft IT21 Statistical Analysis

Marker	Hair 2-3	Hair 2-3, Cauc	Hair 2-3, AA	Hair 4-3	Hair 4-3, Cauc	Hair 4-3, AA
AC004027	IN	0.493213309	0.497574317	NN	0.311645772	0.216386971
Ya5-MLS26	IN	0.465642379	0.25395411	IN	0.465642379	0.25395411
CHR20-79712	NN	0.269794514	0.478230737	IN	0.499245923	0.426623103
Ya5NBC216	N	0.582524272	0.800995025	II	0.50230936	0.359403233
Yb8NBC106	IN	0.494297295	0.489591842	IN	0.494297295	0.489591842
Yc1RG148	NN	0.50230936	0.221040321	NN	0.50230936	0.221040321
Yb8NBC13	NN	0.4013161	0.602361328	NN	0.4013161	0.602361328
Ya5ac2265	I	1.45631068	0.791044776	II	0.530210199	0.156437959
Ya5-MLS09	NN	0.333702517	0.587015173	IN	0.487934772	0.358307963
Yb8AC1141	I	1.223300971	0.467661692	IN	0.475068338	0.358307963
TARBP1	IN	0.487934772	0.404160788	IN	0.487934772	0.404160788
Ya5ac2305	IN	0.492635969	0.422761813	IN	0.492635969	0.422761813
ALU-HS4.69	I	0.766990291	0.63681592	NN	0.380078235	0.464567709
Ya5NBC51	I	1.029126214	1.189054726	IN	0.499575832	0.482129155
Ya5ACA1766	II	0.374116316	0.531230663	II	0.374116316	0.531230663
Yb8NBC120	IN	0.482986144	0.481176209	II	0.166273918	0.356426821
Yb9NBC10	NN	0.311645772	0.116141927	I	0.883495146	1.31840796
Ya5NBC102	NN	0.339334527	0.368406723	IN	0.486379489	0.477116903
Sb19.12	IN	0.426465737	0.477116903	NN	0.478514704	0.368406723
Yb8NBC148	II	0.74663022	0.299497537	II	0.74663022	0.299497537
Total frequency		2.46828E-06	1.38552E-07		6.23338E-07	5.54807E-09
		1 in 405 thousand Cauc	**1 in 7.2 million AA**		**1 in 1.6 million Cauc**	**1 in 180 million AA**

Using in-house database, 2pq & p^2 (2p for drop-out markers)

Hair Shaft Study Conclusions

- In spite of hair shafts exhibiting both LCN and degradation, 50% of the samples produced interpretable profiles
- Obtaining a profile was largely dependent on DNA quantity AND Degradation Index.
- Hair shafts processed with InnoTyper™ 21 provided usable genotype data with statistical power comparable to or better than mtDNA
- Typically, a forensic laboratory will not process hair shaft samples; or will send to a mtDNA lab
- InnoTyper™ provides a way for forensic labs to process hair shafts using existing platforms

InnoTyper™ 21 and Missing Persons

Data from Dixie Peters at UNT Center for Human ID – Molecular and Medical Genetics Department (see Dixie Peters' talk #B133 on Friday, February 20, 2015 at 11:25 AM)

- Sensitivity study performed with skeletal remains previously tested with Identifiler Plus and mtDNA
- MP human remains with no previous successful profile data using autosomal STR and mtDNA

InnoTyper™ 21 Sensitivity Study

Three skeletal samples chosen and eight different concentrations of each sample were amplified (500pg, 250pg, 125pg, 62.5pg, 31.25pg, 15.63pg, 7.8pg, 3.9pg)

Sample	AC004027	Ya5-MLS26	CHR20-79712	Ya5NBC216	Yb8NBC106	Yc1RG148	Yb8NBC13	Ya5ac2265	Ya5-MLS09	Yb8AC1141	TARBP1	AMEL	Ya5ac2305	ALU-HS4.69	Ya5NBC51	Ya5ACA1766	Yb8NBC120	Yb9NBC10	Ya5NBC102	Sb19.12	Yb8NBC148
UNT1_3.9		N	300	I		I,N	N	I,N	N	N	I	66		I	N						
UNT1_7.81	I,N	N	I,N	I			N	I,N	N	I	I	112		I	N		N			84	
UNT1_15.63	I,N	N	I,N	I	I,N	I,N	N	184	N	I	I	X,Y	I	I	N	I	N	N	N,I	69	I
UNT1_31.25	I,N	N	I,N	I	I,N	I,N	N	I,N	N	I	I	X,Y	I	I	N	I	N	N	N,I	I,N	I
UNT1_62.5	I,N	N	I,N	I	I,N	I,N	N	I,N	N	I	I	X,Y	I	I	N	I	N	N	N,I	I,N	I
UNT1_125	I,N	N	I,N	I	I,N	I,N	N	I,N	N	I	I	X,Y	I	I	N	I	N	N	N,I	I,N	I
UNT1_250	I,N	N																	N,I	I,N	I
UNT1_500	I,N	N																	N,I	I,N	I
UNT6_3.9	N	N																			I
UNT6_7.81	N	N																	I		
UNT6_15.63	N	N																	I	124	I
UNT6_31.25	N	N																	I	I,N	I
UNT6_62.5	N	N																	I	I,N	I
UNT6_125	N	N	I,N	I,N	I,N	N	N	I	I,N	I	I	X	I,N	N	I	I,N	N	N	I	I,N	I
UNT6_250	N	N	I,N	I,N	I,N	N	N	I	I,N	I	I	X	I,N	N	I	I,N	N	N	I	I,N	I
UNT6_500	N	N	I,N	I,N	I,N	N	N	I	I,N	I	I	X	I,N	N	I	I,N	N	N	I	I,N	I
UNT7_3.9	N	I	187		N	N	153	I,N	N		I	X,Y		112	96	70	75				I
UNT7_7.81	N	I	I,N	I,N	N	N	252	352	N	I	I	237	I,N	154	N,I	73	52		I		I
UNT7_15.63	N	I	I,N	664	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	193	I,N	I,N	I		I
UNT7_31.25	N	I	I,N	I,N	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	I,N	I,N	I,N	I	I	I
UNT7_62.5	N	I	I,N	I,N	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	I,N	I,N	I,N	I	I	I
UNT7_125	N	I	I,N	I,N	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	I,N	I,N	I,N	I	I	I
UNT7_250	N	I	I,N	I,N	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	I,N	I,N	I,N	I	I	I
UNT7_500	N	I	I,N	I,N	N	N	I,N	I,N	N	I	I	X,Y	I,N	I,N	N,I	I,N	I,N	I,N	I	I	I

3 skeletal remains produced a full profile at 31 picograms (50 RFU min threshold)

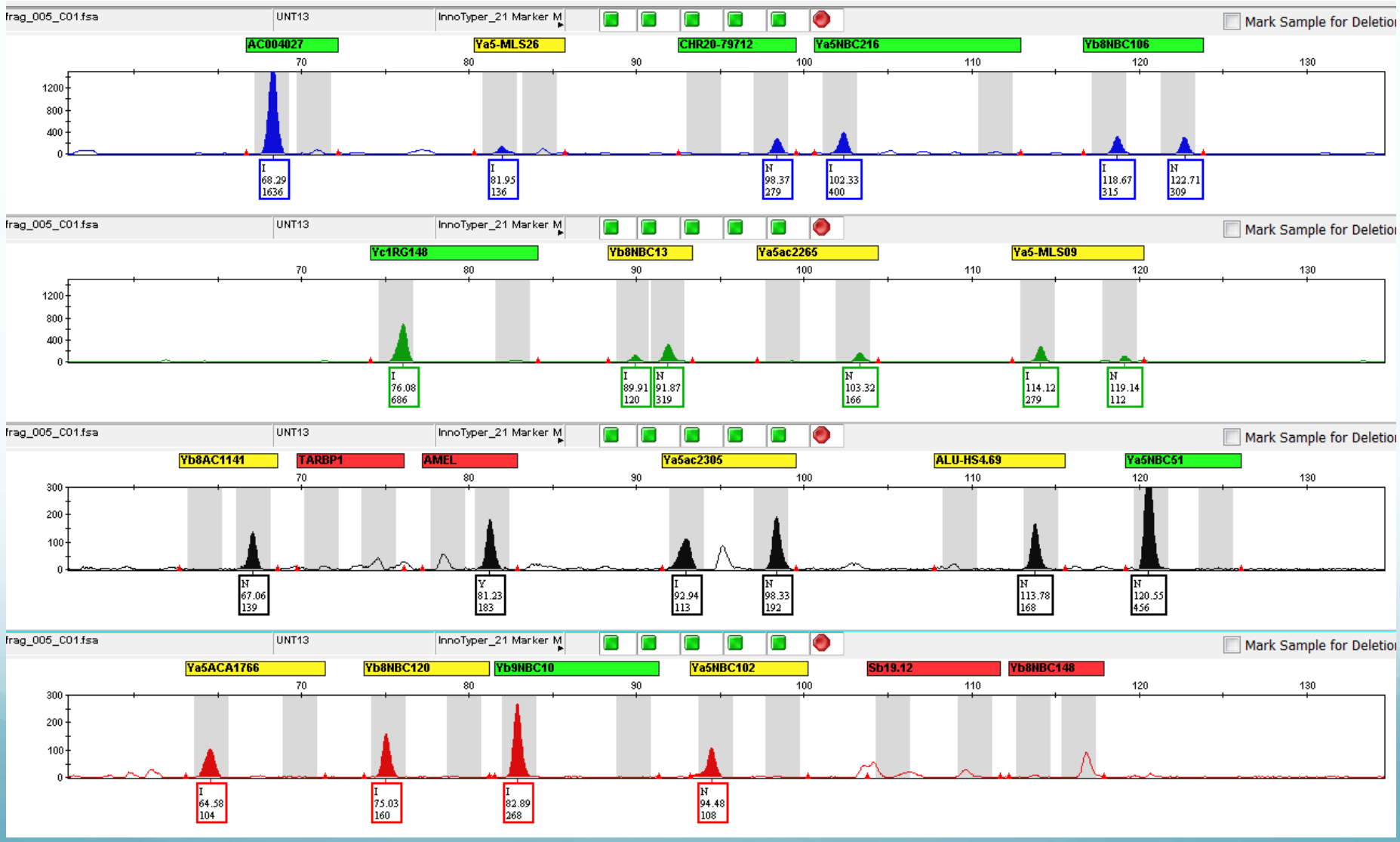
	Lowest concentration produced a full profile
	RFU of surviving sister allele
	Complete allele dropout observed
	Discordance
50 RFU threshold used	

InnoTyper™ 21 and Missing Persons

Sample Name	Quant	uL amp'd	STR Information	Mito Information	IT21 Information	Avg IT21 RFU	Genotype Frequency
UNT 11	UND	10.4	Profiler/Cofiler increased cycles, NR	NR	4 locus partial	779	1 in 226 Cauc 1 in 4,656 AA
UNT 12	UND	10.4	Profiler/Cofiler increased cycles, NR	NR	NR (1 locus)	544	
UNT 13	UND	10.4	Profiler/Cofiler increased cycles, NR	Inconclusive results	17 locus partial	302	1 in 14 billion Cauc 1 in 254 million AA
UNT 14	UND	10.4	Profiler/Cofiler increased cycles, NR	NR	NR	-	
UNT 15	UND	10.4	Profiler/Cofiler increased cycles, NR	NR	6 locus partial	271	1 in 953 Cauc 1 in 661 AA
UNT 16	UND	10.4	Profiler/Cofiler increased cycles, NR	Reportable (16024-16386; 52-302; 316-399)	2 locus partial	223	
UNT 17	UND	10.4	Profiler/Cofiler increased cycles, NR	Reportable (16001-16386; 72-399)	2 locus partial	260	

Three of seven remains yielded usable genotype data with InnoTyper™ 21

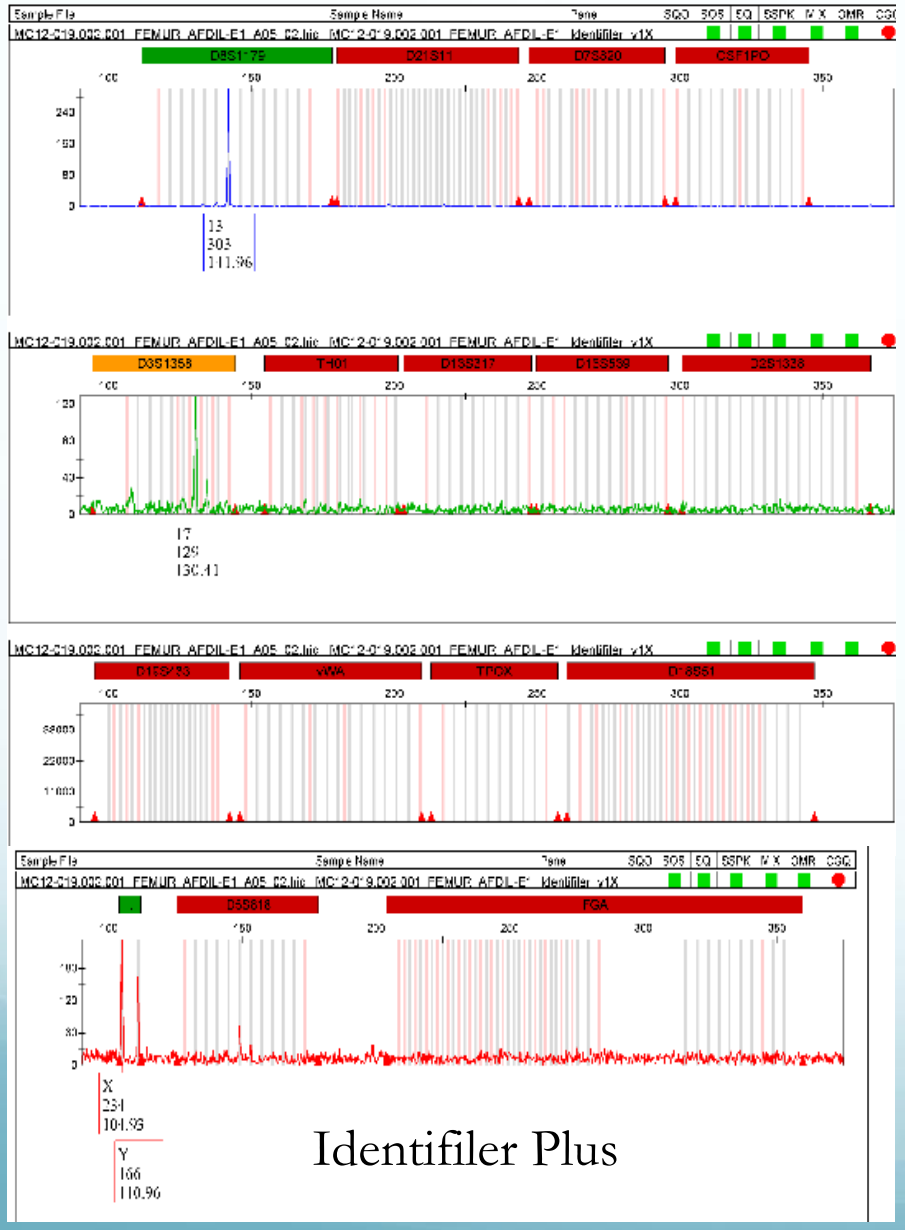
Human Remains Result – UNT13

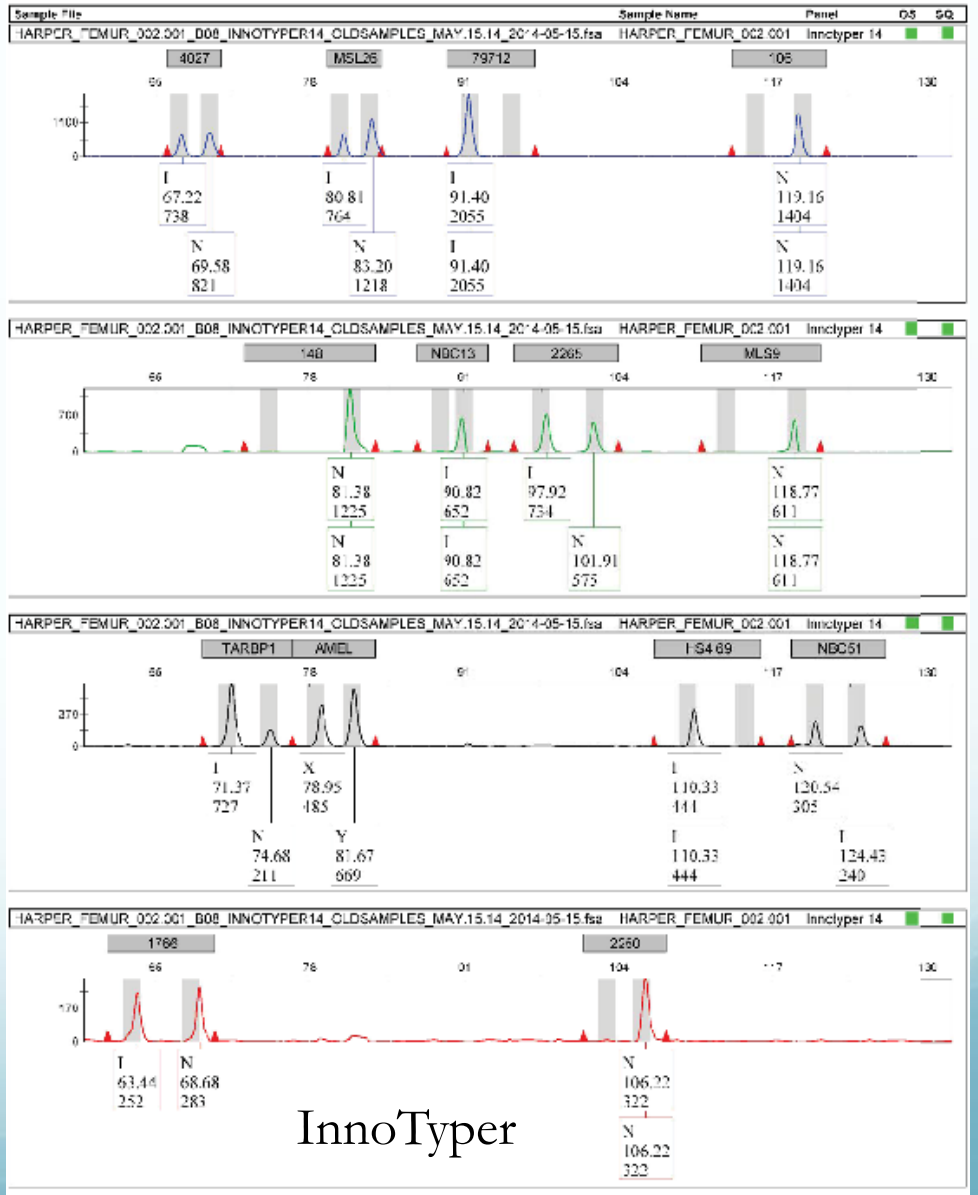


Missing Persons Study Conclusions

- InnoTyper™ 21 can be used in the analysis of human remains
- InnoTyper™ proves useful even in cases where previous STR and mtDNA tests were unsuccessful
- Highly sensitive method(optimal target amount is ~250 pg but can get full profiles with as low as 31 picograms)
- High power of discrimination

InnoTyper™ and historical remains





InnoTyper™ 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 CE methods.

Conclusions

- Next-generation (NG) systems are now available to improve sample processing and profile success in forensic labs.
- Quantification & Degradation Assessment Kit InnoQuant™ 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 InnoTyper™21 data demonstrates the ability to obtain successful DNA profiles from challenging samples, such as skeletal remains, historical remains and cut hair shafts that all previously failed to produce STR data.

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Thank You