

# UNT

# HEALTH<sup>™</sup> SCIENCE CENTER



## Utility of a Novel and Sensitive DNA Multiplex for Highly Degraded Missing Persons Samples

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# Commercial Interest

- This presentation is for scientific and educational purposes only and does not promote any company products, directly or indirectly.
- No financial interest in the following products and company:
  - Quantifiler<sup>®</sup> Duo, 7500 Real-Time PCR System, Identifiler<sup>®</sup> Plus, MiniFiler<sup>™</sup>, Profiler Plus<sup>®</sup>, Cofiler<sup>®</sup>, dRhodamine Cycle Sequencing kit, 3130xL Genetic Analyzer, SDS and HID software, GeneMapper<sup>®</sup> ID-X, Sequencher<sup>™</sup>, PowerPlex<sup>®</sup> 5-dye matrix standards, CC5 ILS, InnoGenomics<sup>®</sup>, Innotyper<sup>™</sup> 14, InnoTyper<sup>™</sup> 21

# STRs: Current Standard

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- Robust and reliable genotyping with relatively high quantity and quality samples
- Limitations
  - High mutation rates
  - Degraded samples
    - Amplicon sizes range from 100-400bp
    - Amplicons <200bp are ideal for degraded samples

# Alternatives to STRs

- Bi-allelic markers
  - SNPs
    - No size difference between alleles
  - INDELS
    - Insertion of bases (1-1000's)
  - INNULS
    - Variation on INDELS
    - 2 states: Insertion (**IN**) or null alleles (**NUL**)
    - Insertions are retrotransposable elements (REs)

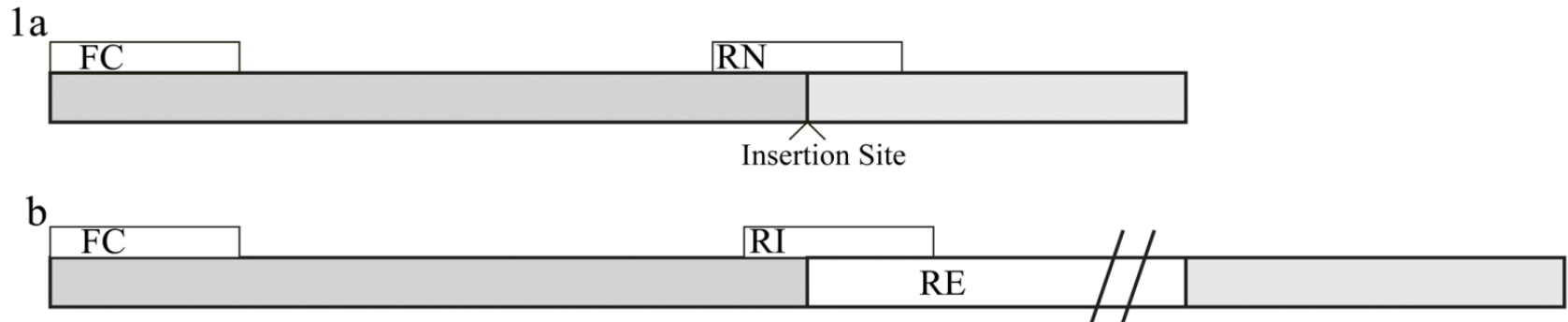
# INNULs

- Transposable elements that move by way of RNA intermediate
- Two categories:
  - Long interspersed elements (LINEs)
    - 1-6.5kb in length
    - 5-10% of genome
  - Short interspersed elements (SINEs) (primarily Alu)
    - 300bp in length
    - 10% of genome

# INNULs

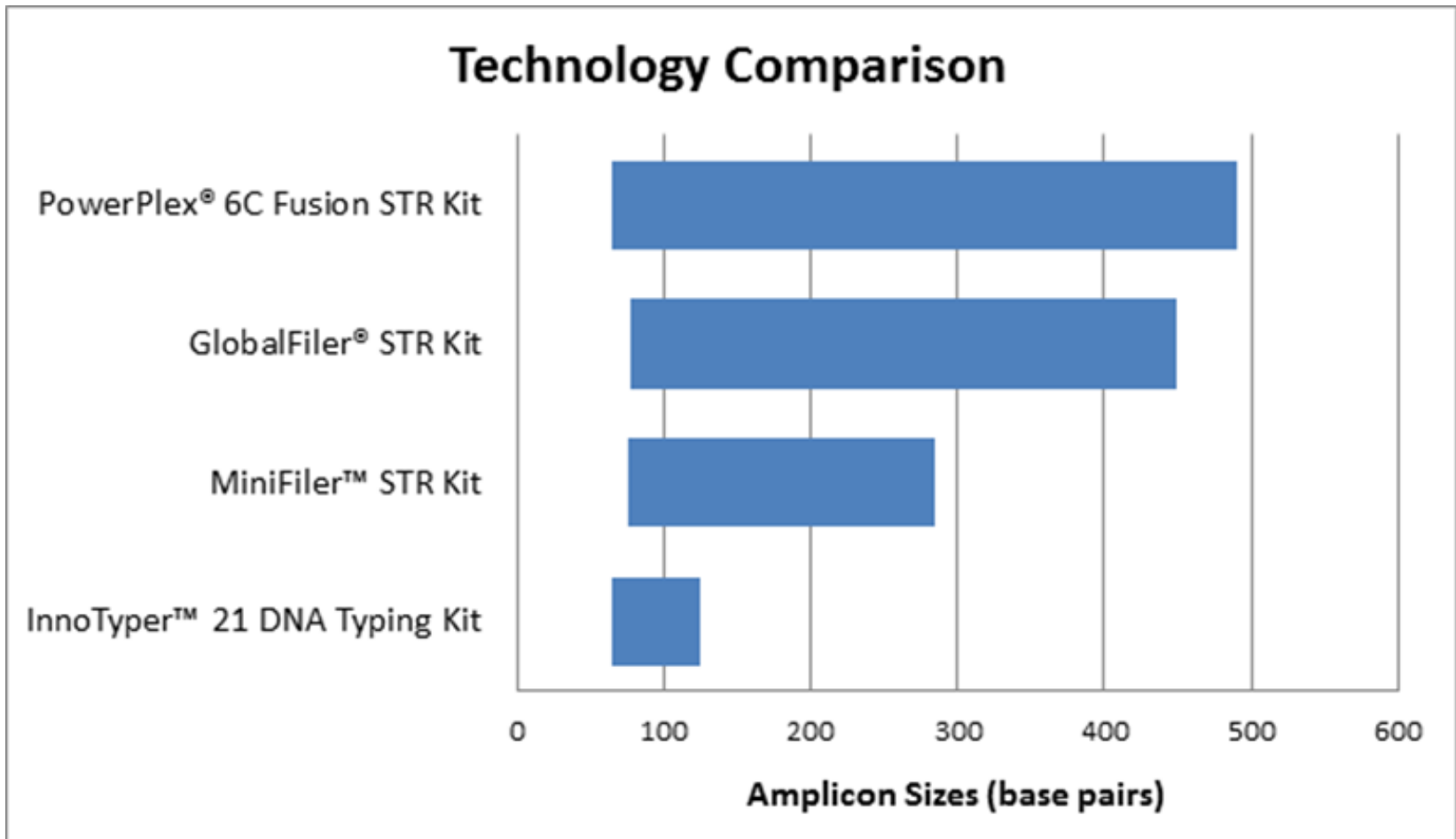
- Advantages:
  - Stable polymorphisms
  - Low mutation rate
  - Identical by descent
- Disadvantages:
  - Size difference between null allele and an insertion element is large
  - Preferential amplification of smaller allele

# Novel Primer Design



- Common forward primer (FC) used for both insertion and null alleles
- A 'null-specific' reverse primer (RN) will only bind if insertion is not present
- A retrotransposable element (RE) disrupts annealing of RN
- An 'insertion-specific' primer (RI) will bind only if insertion is present
- Allows for amplification of small amplicons (60-125bp)

# Amplicon Size Comparison





# Center for Human Identification

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- Missing Persons Laboratory
  - Over 700 unidentified remains samples per year
  - Skeletal remains aged from <1 year to >80 years

# InnoTyper 21

- Initially tested InnoTyper14
  - 0.5ng target: overblown
- InnoTyper21 testing
  - **Sensitivity**
  - **Challenging skeletal remains** (mtDNA obtained, little to no STR results)
  - **Extremely challenging skeletal remains** (little to no mtDNA results, no STR results)

# InnoTyper21 Protocol

- 25 $\mu$ l reactions
  - InnoTyper™ Primer Mix, InnoTyper™ Master Mix, IGT DNA polymerase
  - Allows for 10.4 $\mu$ l of template
  - 32 cycles
  - ~2.5 hours cycling time

# InnoTyper21 Protocol

- Run conditions
  - 5-dye matrix standard
  - 1 $\mu$ l of PCR product
  - CC5 ILS
  - No allelic ladder
  - 3130xL Genetic Analyzer
  - POP6
  - 10 sec/3kV injection
- Analysis: GM ID-X
  - 50 and 100 RFU thresholds

# InnoTyper21: Sensitivity

- Tested 8 concentrations: 500pg, 250pg, 125pg, 62.5pg, 31.25pg, 15.6pg, 7.8pg, and 3.9pg

Sample	Type	Estimated Age	Quant Duo (ng/ $\mu$ l)
UNT 1	teeth	2.5 years	0.201
UNT 6	vertebra	< 1 year	0.273
UNT 7	rib	< 1 year	0.0585

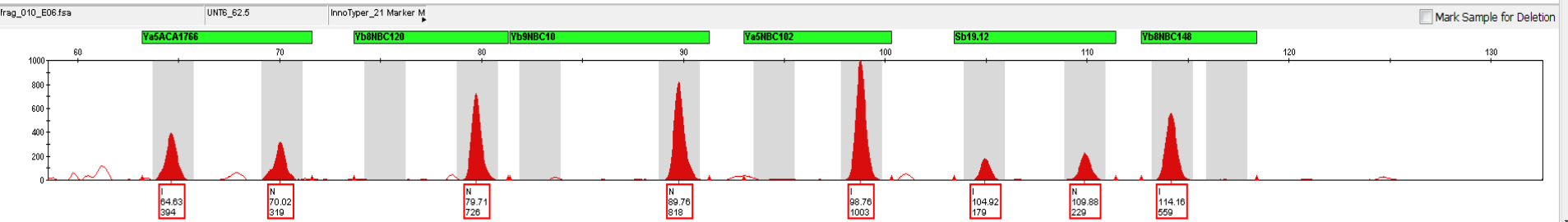
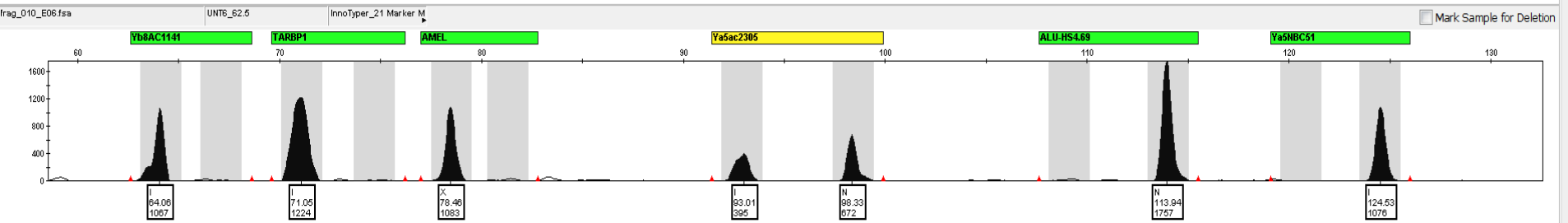
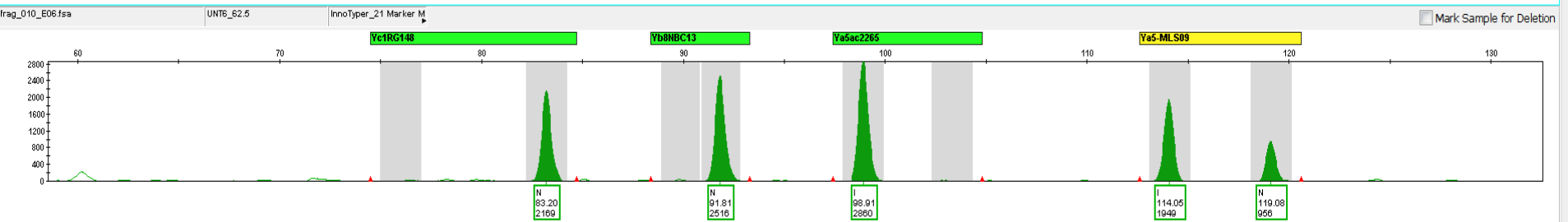
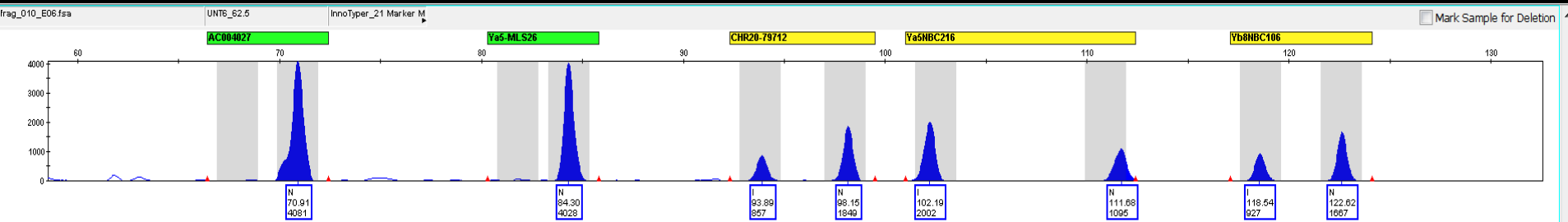
- 15 loci plus amelogenin with Identifiler Plus
- HV1 and HV2 mitochondrial DNA profiles

# InnoTyper21: Sensitivity

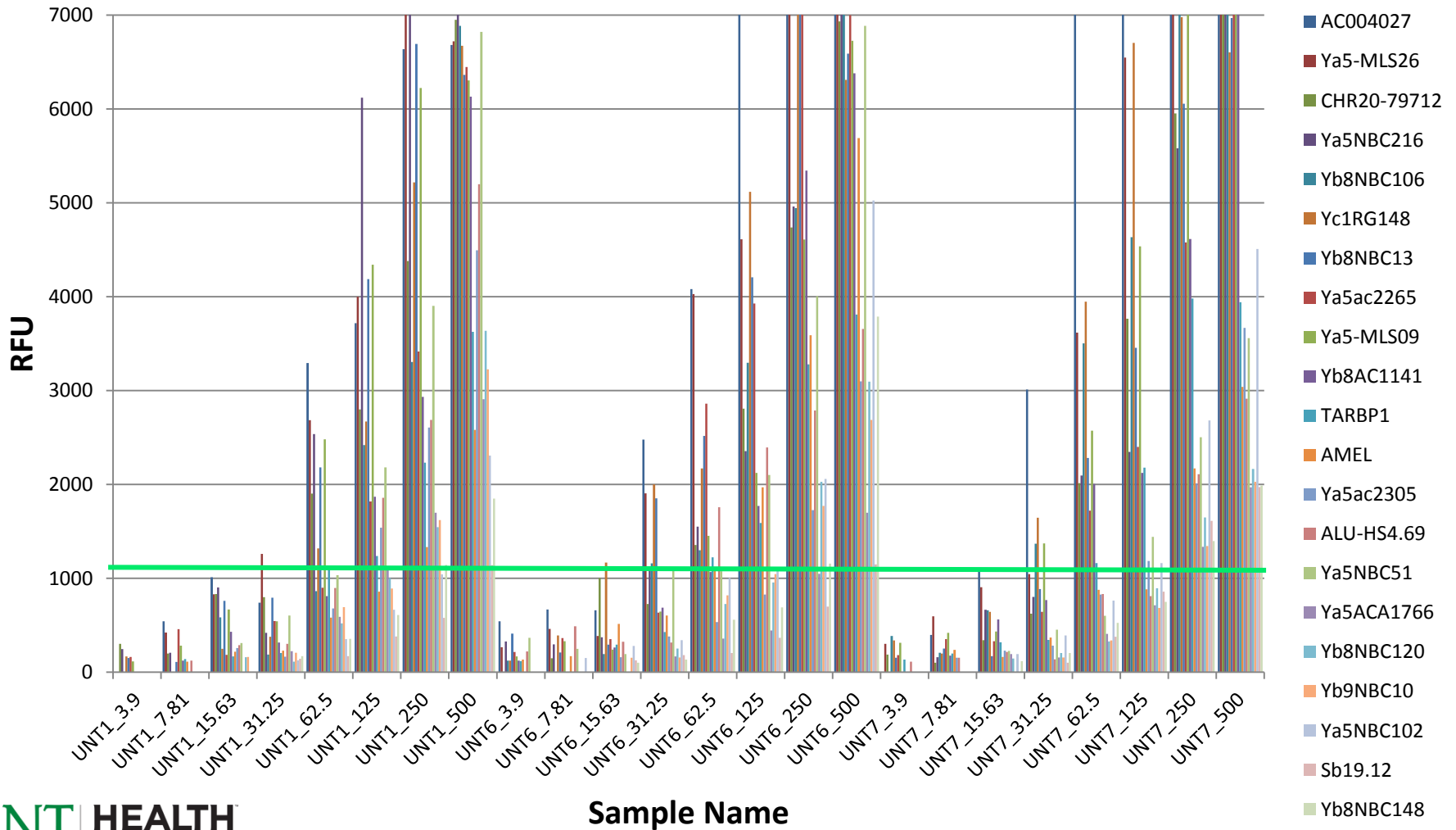
Sample	AC004027	Ya5-MLS26	CHR20-79712	Ya5NBC216	Yb8NBC106	Yc1RG148	Yb8NBC13	Ya5ac2265	Ya5-MLS09	Yb8AC1141	TARBP1	AMEL	Ya5ac2305	ALU-HS4.69	Ya5NBC51	Ya5ACA1766	Yb8NBC120	Yb9NBC10	Ya5NBC102	Sb19.12	Yb8NBC148
UNT1_500	I,N	N	I,N	I	I,N	I,N	N	I,N	N	I	I	X,Y	I	I	N	I	N	I,N	N,I	I,N	I
UNT1_250	I,N	N	I,N	I	I,N	I,N	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_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	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
UNT1_15.63	I,N	N	I,N	I	I,N	I	N	N	N	I	I	Y	I	I	N		N	N			
UNT1_7.81	I,N	N	I,N	I			N	I	N	I	I	X		I							
UNT1_3.9		N	I	I		I,N	N	I	N												
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
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_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_62.5	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_31.25	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	N	I
UNT6_15.63	N	N	I,N	N	I,N	N	N	I	I,N	I	I	X	I,N	N	I			N	I	I	I
UNT6_7.81	N	N	I	I,N		N	N	I	N			X		N	I				I		
UNT6_3.9	N	N		N	I,N	N	N	I	I,N	I	I	X		N	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
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_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_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_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_15.63	N	I	I,N	I	N	N	I,N	I,N	N	I	I	X,Y	I,N	I	I	I	I,N		I		I
UNT7_7.81	N	I	I	I,N	N	N	I	I	N	I	I	X	I,N	N							
UNT7_3.9		I	N		N	N	N	I,N	N		I			I							

Lowest concentration produced a full profile  
 Sister allele dropout observed  
 Complete allele dropout observed

100 RFU threshold used

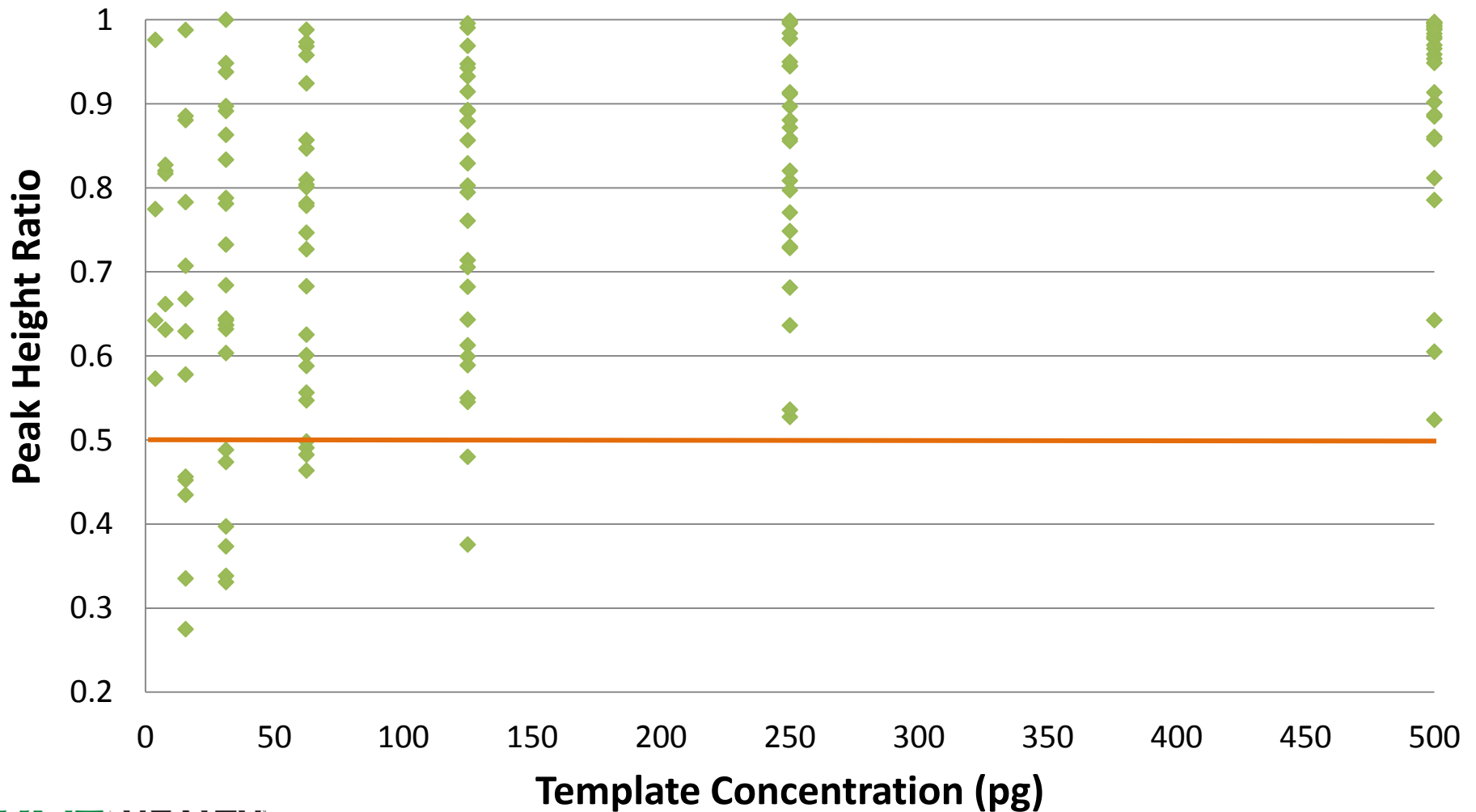


# InnoTyper21: Sensitivity





# InnoTyper21: Sensitivity



# InnoTyper21: Sensitivity

- Allelic drop-out
  - 27 observations (13% of heterozygous loci ampmed)
  - Surviving sister allele
    - Range of RFUs: 101-664
    - Average RFU: 232
    - Median RFU: 193
- No results: 68 observations (13% of 504 loci total)
- Amelogenin concordant with previous ID Plus data
- Range of PCR product sizes: 64.03-124.56bp

# Skeletal Remains, set 1

Sample Name	Sample Type	Estimated Age	Quantity Amplified (pg)	Previous Results		InnoTyper21 Loci		
				Mito	Number of STR Loci	Above 100 RFU	ADO	IMB (<0.5)
UNT 18	femur	23 years	13.9	HV1 & HV2	IC = 0	19	3	3
UNT 19	metacarpal	42 years	5.0	HV1 & HV2	IC = 0	18	3	3
UNT 20	femur	unknown	UND	HV1 & HV2	IC = 0	16	3	0
UNT 21	femur	unknown	UND	HV1 & HV2	IC = 0	5	1	0
UNT 22	humerus	unknown	UND	HV1 & HV2	IC = 4, MF = 0, Total = 4	20	3	2
UNT 23	femur	unknown	16.2	HV1 & HV2	IC = 6, MF = 7, Total = 11	20	0	0
UNT 24	tibia	41 years	UND	HV1 & HV2	IC = 2, MF = 5, Total = 7	20	1	2
UNT 25	rib	5 years	UND	HV1 & HV2	IC = 5, MF = 2, Total = 6	18	4	4
UNT 26	tibia	23 years	39.8	HV1 & HV2	IC = 5, MF = 5, Total = 8	21	0	3

Amelogenin data concordant with previous results

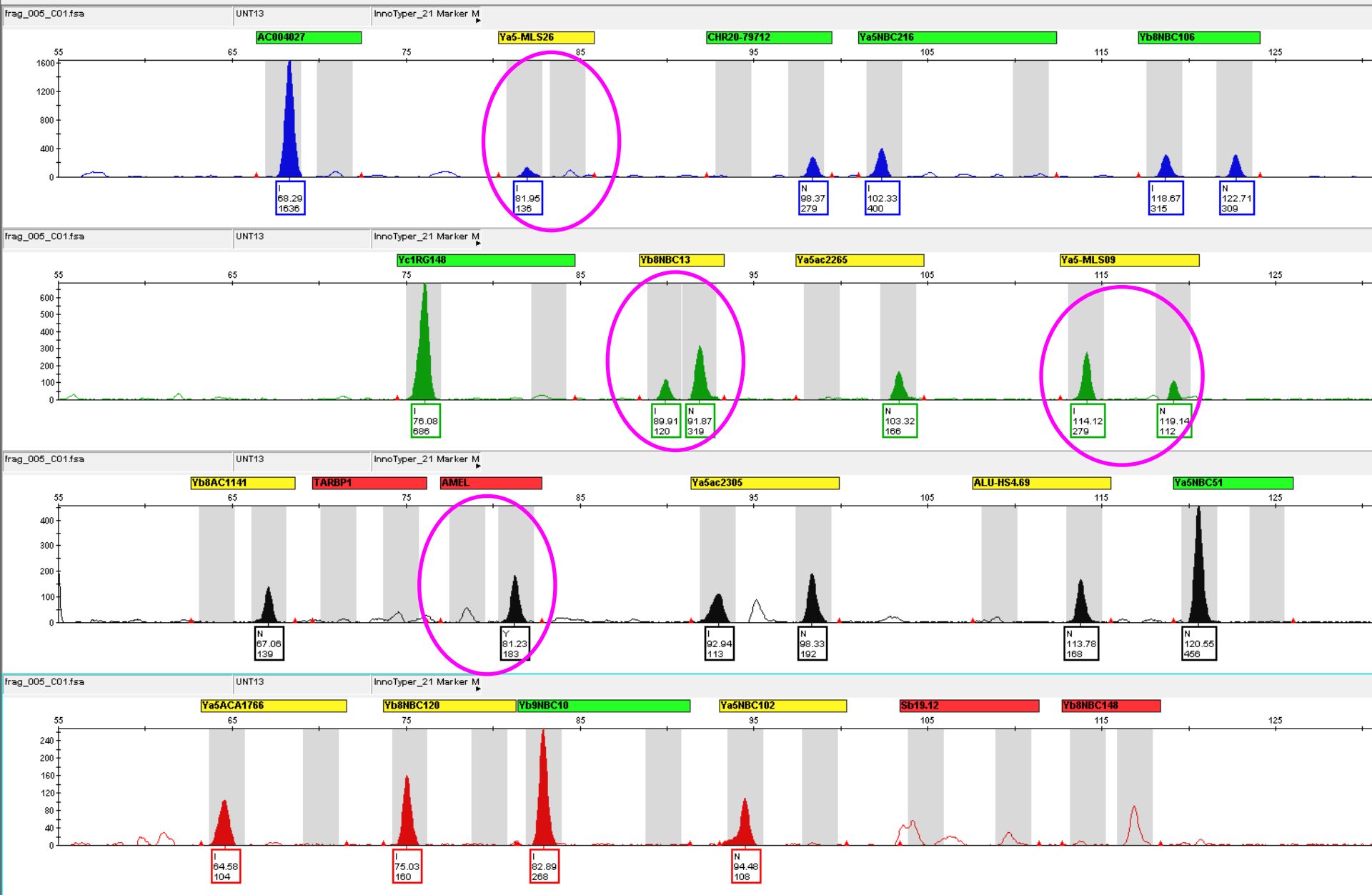
RFU range: 100-7762; AVG RFU: 535



UNT 26, 39.8pg

# Skeletal Remains, set 2

Sample Name	Sample Type	Estimated Age	Quantity Amplified (pg)	Previous Results		InnoTyper21 Loci	
				Mito	Number of STR Loci	Above 100 RFU	AVG RFU
UNT 11	tooth	65-90 years	UND	NR	IC = 0	4	779
UNT 12	tooth	65-90 years	UND	NR	IC = 0	1	544
UNT 13	tooth	65-90 years	UND	INC	IC = 0	17	302
UNT 14	tooth	65-90 years	UND	NR	IC = 0	0	NA
UNT 15	tooth	65-90 years	UND	NR	IC = 0	6	271
UNT 16	tooth	65-90 years	UND	HV1 & HV2	IC = 0	2	223
UNT 17	tooth	65-90 years	UND	HV1 & HV2	IC = 0	2	305



UNT 13

# Discriminatory Power

Population	N	Combined RMP
African American	47	$2.52 \times 10^{-8}$
Asian American	44	$2.64 \times 10^{-7}$
Caucasian	45	$1.94 \times 10^{-8}$
Southwest Hispanic	45	$3.09 \times 10^{-8}$

# Summary

- Sensitivity study
  - 31.25-125pg produced full profiles
- Skeletal Remains, sets 1 and 2

	Previous Results	InnoTyper Results
<b>No STRs</b>	<b>11</b>	<b>7 (64%)</b>
<b>Reportable STRs</b>	<b>5</b>	<b>5 (100%)</b>
<b>TOTAL</b>	<b>16</b>	<b>12 (75%)</b>

- Multiple amplifications/consensus approach may be needed to assess allele drop-out



# Summary

- **INNULS**
  - Stable, well-characterized markers
  - Useful for kinship analysis
  - High power of discrimination
- **InnoTyper21 kit**
  - Sensitive
  - Some success with degraded and aged bone samples
  - Subject to similar STR kit problems (pull-up, split peaks, and artifacts)
  - Simplified analysis

# Acknowledgements

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