

# UNT

# HEALTH<sup>TM</sup> SCIENCE CENTER

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## Use of InnoTyper21 with Highly Degraded Missing Persons Samples

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June 29, 2016

# 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 in STR kits
    - Amplicons <200bp are ideal for degraded samples

# Alternatives to STRs

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

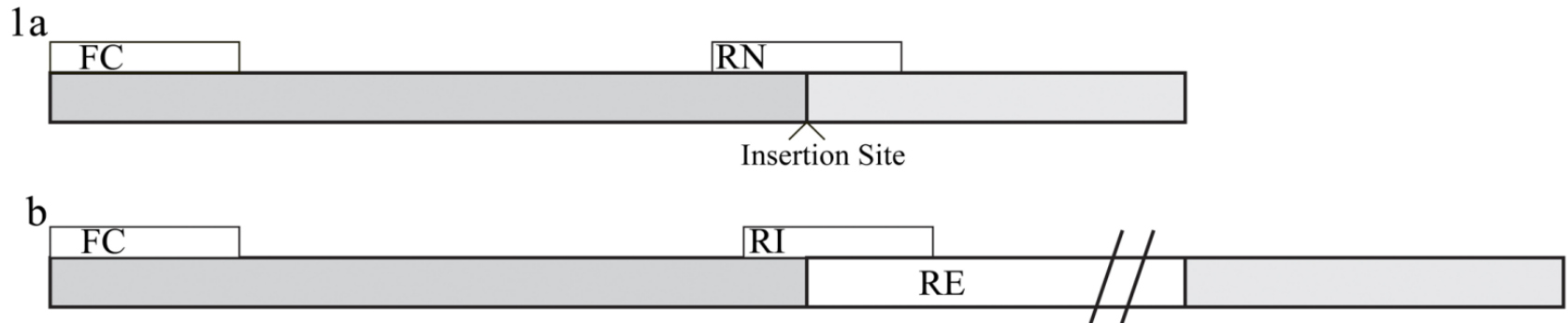
# INNULs

- 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

# IGT 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)

# UNTHSC Center for Human Identification

- Missing Persons Laboratory
  - ~700 unidentified remains samples per year
  - Skeletal remains aged from <1 year to >80 years
  - STRs, Y-STRs, and mitochondrial DNA
- Standard method: Identifiler Plus 28 & 29 cycles
- Enhanced detection method: Increased cycles (P/C 32), amplifications in triplicate, consensus profile
  - 44% of samples require increased cycles (IC)
  - 39% of samples require Minifiler

# InnoTyper 21

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## **1. Sensitivity**

**2. Challenging skeletal remains** (mtDNA obtained, little to no STR results)

**3. 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 (UPDATE: 16 $\mu$ l)
  - 32 cycles (UPDATE: 31)
  - ~2.5 hours cycling time

# InnoTyper21 Protocol

- Run conditions
  - 5-dye matrix standard (UPDATE: includes kit specific matrix)
  - 1µl of PCR product
  - CC5 ILS (UPDATE: includes kit specific ILS)
  - No allelic ladder (UPDATE: includes 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/μl) |
|--------|----------|---------------|-------------------|
| 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

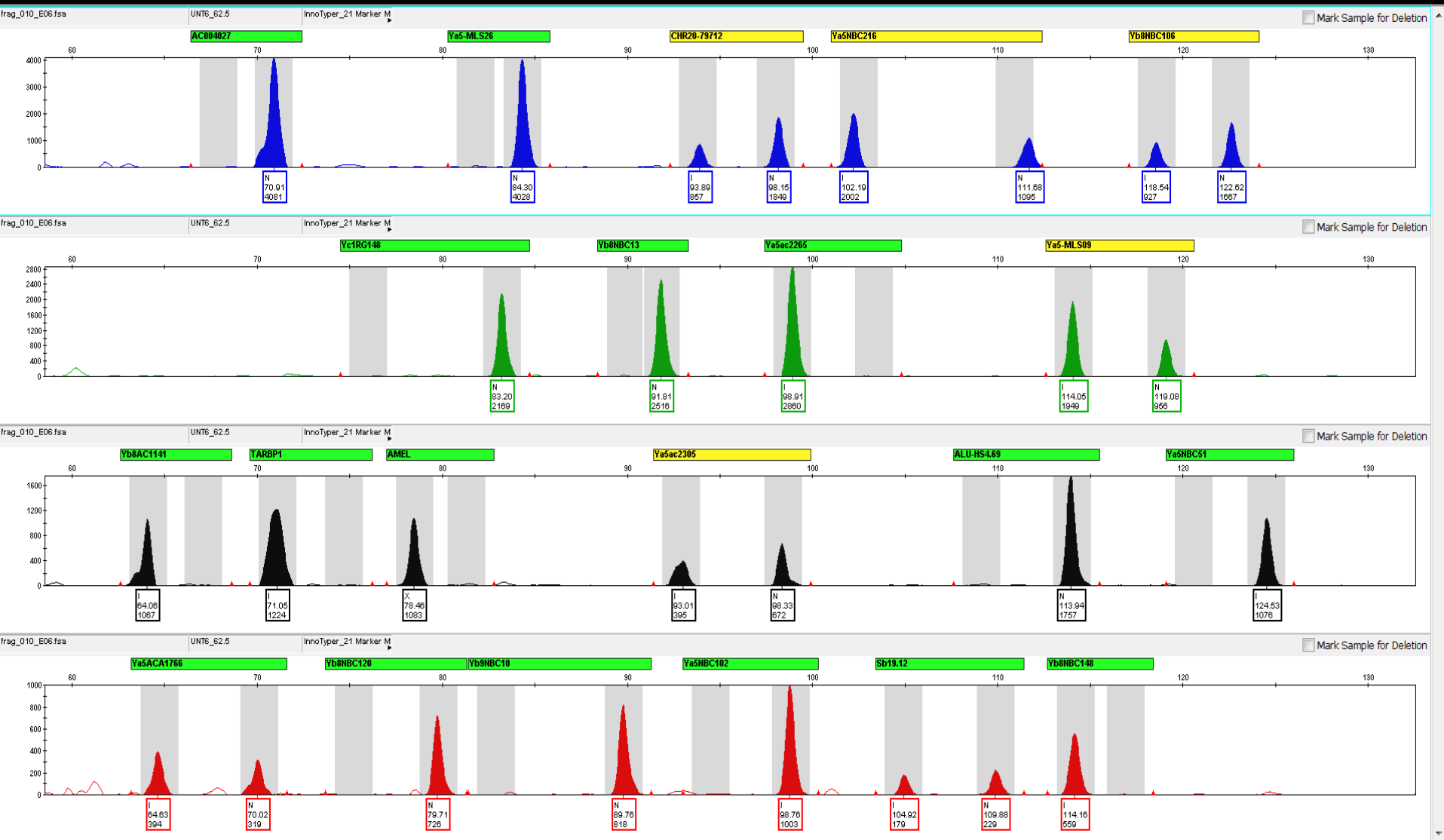
| Sample     | AC004027 | Ya5-MLS26 | CHR20-79712 | Ya5NBC216 | Yb8NBC106 | Yc1RG148 | Yb8NBC13 | Ya5ac2265 | Ya5-MLS09 | Yb8AC1141 | TARBP1 | AMEL | Ya5ac2305 | ALU-HS4.69 | Ya5NBC51 | Ya5ACA1766 | Yb8NBC120 | Yb9NBC10 | Ya5NBC102 | Sb19.12 | Yb8NBC148 |
|------------|----------|-----------|-------------|-----------|-----------|----------|----------|-----------|-----------|-----------|--------|------|-----------|------------|----------|------------|-----------|----------|-----------|---------|-----------|
| 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

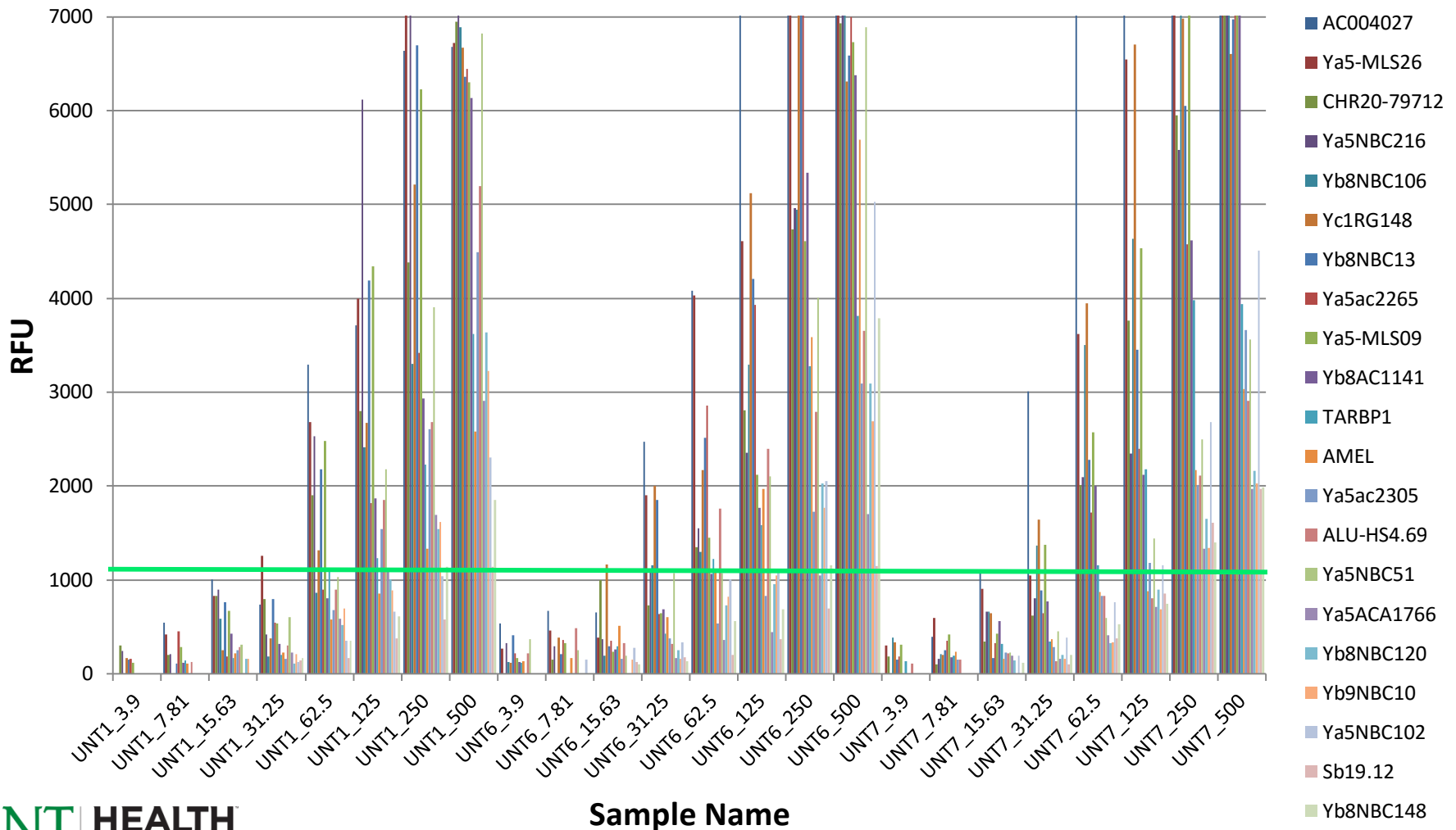
Complete allele dropout observed

100 RFU threshold used

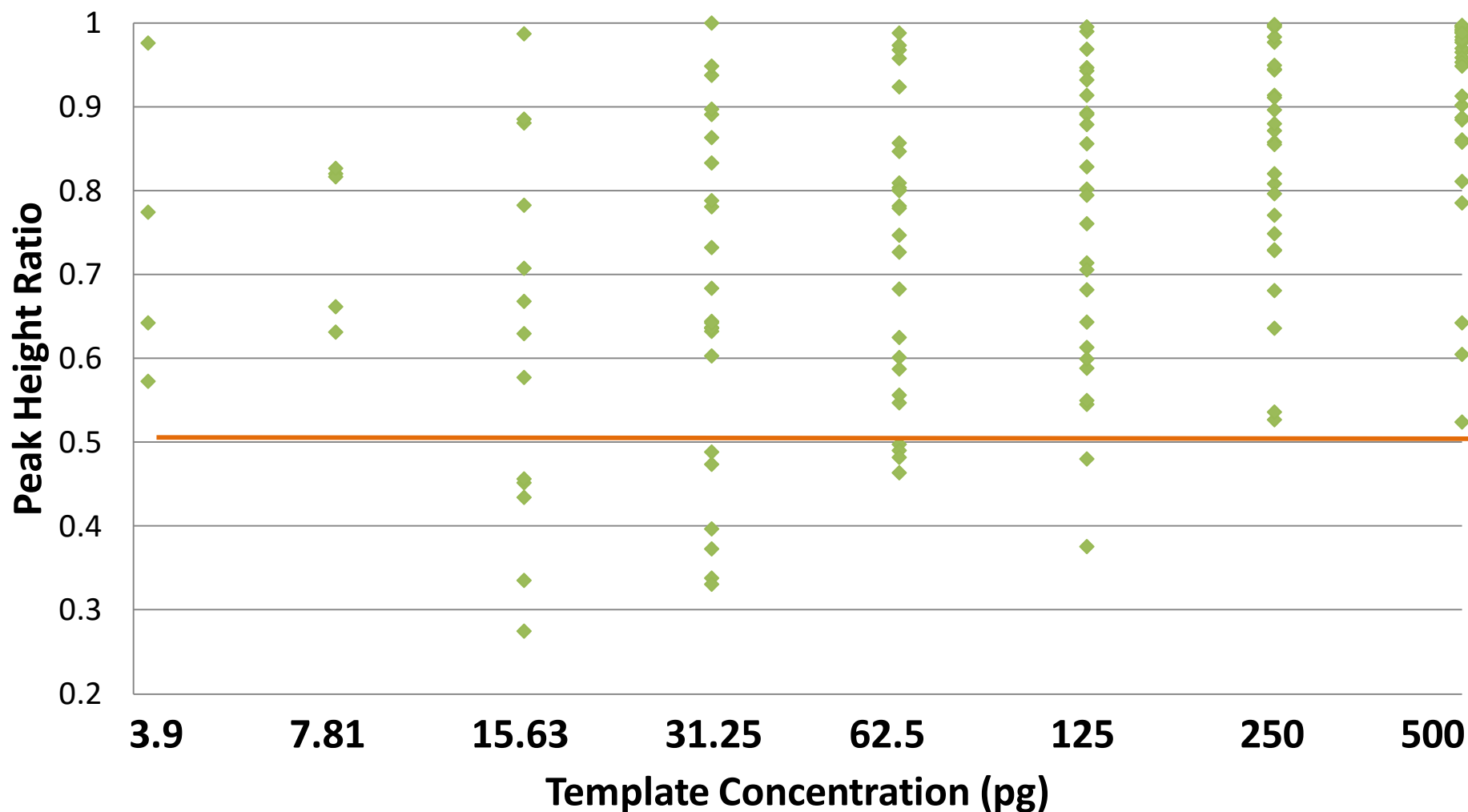


UNT 6, 62.5pg

# InnoTyper21: Sensitivity



# InnoType21: 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    | Allelic Drop-out | PH 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     |

# Skeletal Remains, set 2



4 loci

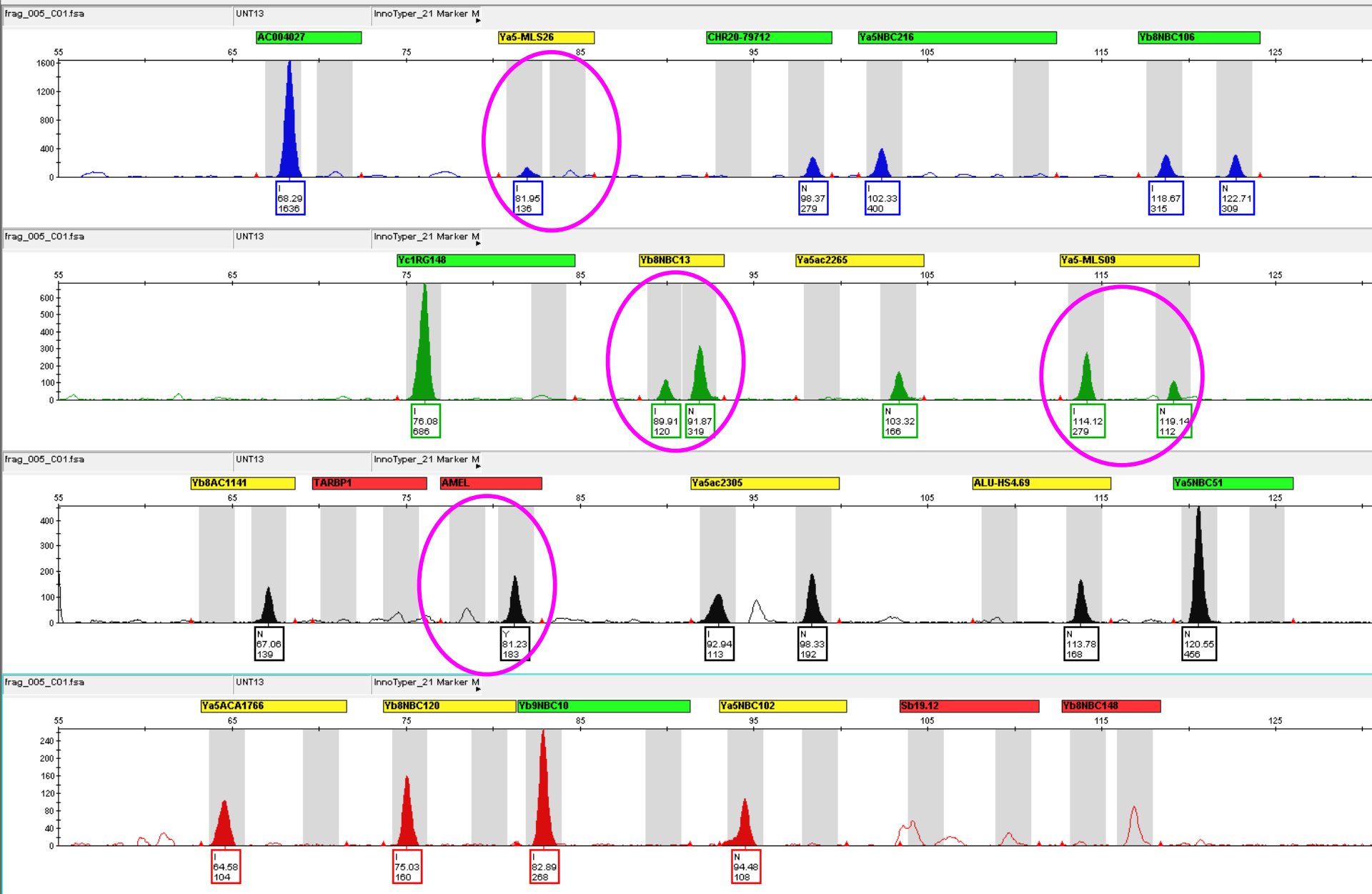


17 loci



2 loci

InnoTyper21 results



UNT 13

# Discriminatory Power of IT21

| 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 |    | InnoTyper21 Results |
|------------------|----|---------------------|
| No STRs          | 11 | 7 (64%)             |
| Reportable STRs  | 5  | 5 (100%)            |
| TOTAL            | 16 | 12 (75%)            |

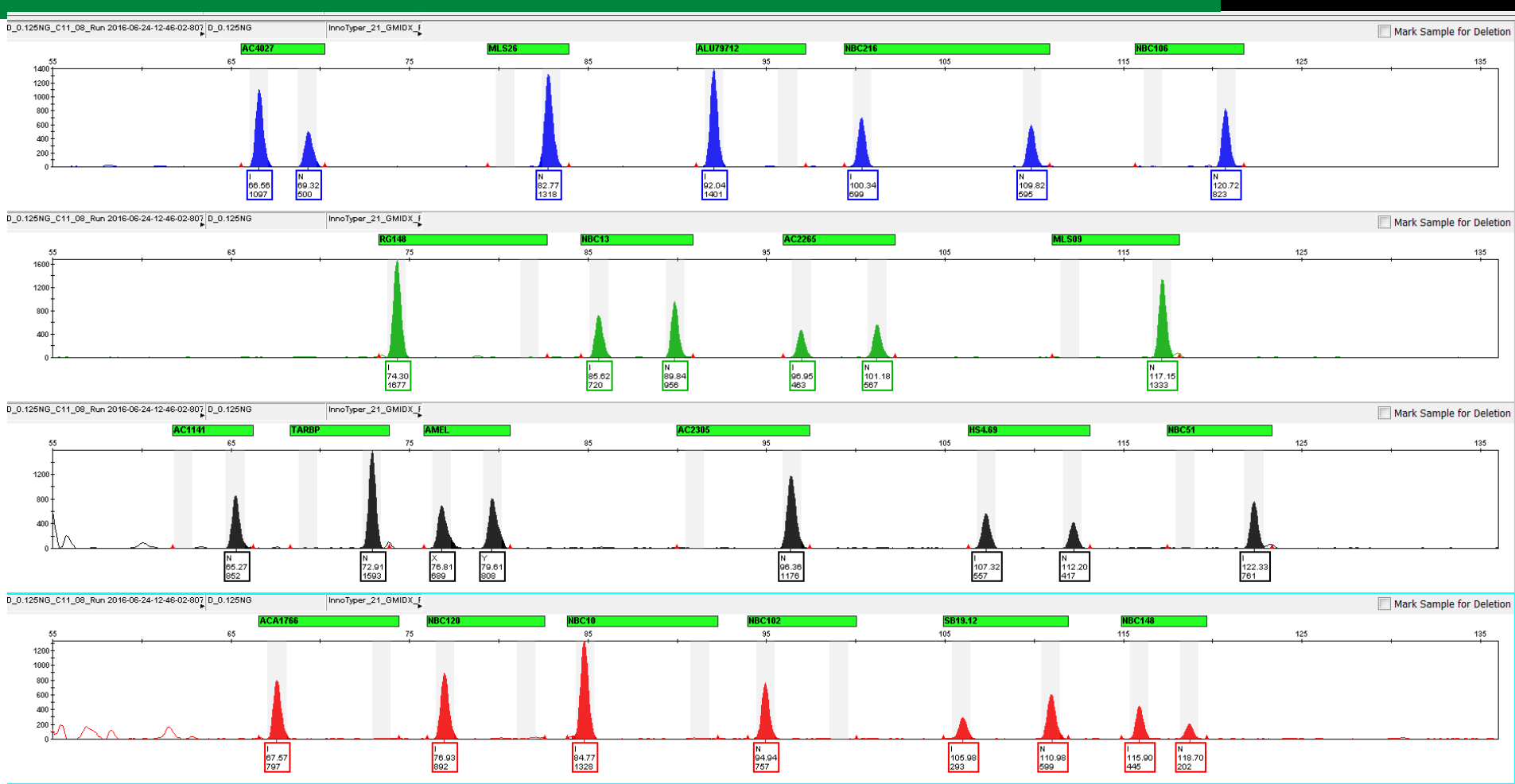
- Multiple amplifications/consensus approach may be needed to assess allele drop-out

# IT21 Internal Validation

- Parameters:
  - 16µl of DNA template
  - IGT 5 dye matrix, ILS-155 internal lane standard, and IGT allelic ladder
  - 3500xL with POP6
  - GM ID-X v1.5



# IT21 Internal Validation



# IT12 Internal Validation

- Peak height ratio averages >87%
- Peak height averages (RFUs):
  - 500pg = 2529
  - 250pg = 1498
  - 125pg = 887

# 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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- University of North Texas Health Science Center
  - Bobby LaRue, PhD
- InnoGenomics Technologies
  - Sudhir Sinha, PhD
  - Gina Pineda Murphy, MS

# Contact information

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