



Technology Transition Workshop | *Thomas Hall, Ph.D.\**

***DNA Mixture Detection,  
Analysis, and Interpretation  
Using the Ibis™ Assays***

**\*Original material used with permission from Leslie D. McCurdy, Ph.D., Federal Bureau of Investigation, DNA Analysis Unit II**

**Additional input gathered from**

- **Bruce Budowle**
- **Constance Fisher**
- **Steven Hofstadler**
- **Alice Isenberg**
- **Thuy-Trang Pennell**
- **Kristin Sannes-Lowery**

# ***Outline***

- **Mitochondrial DNA mixtures**
  - Heteroplasmy – Length and SNP
  - Mixed templates
- **STR mixtures**

# Mitochondrial DNA Mixtures

## What Is a Mixture?

- **Natural**

- **Heteroplasmy**

- **Point/sequence**
- **Length**

- **Situational**

- **Multiple contributors**

Avg. Number of Nucleotide Differences Between Individuals	
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US Caucasians	8
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African Americans	14
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Hispanic	13
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Budowle et al. *Forensic Science International* 1999;103:23-35

# ***Mitochondrial DNA Mixtures***

## ***Current Interpretations***

- **Heteroplasmy – when comparing two profiles with one or more mixed bases**
  - **Common base at each position?**
  - **Common length variants detected?**
  - **Concordant mtDNA types?**
- **Multiple contributors**
  - **Not readily interpretable using current CGE sequencing**

# ***Mitochondrial DNA Mixtures***

## ***Challenges***

- **Heteroplasmy vs. multiple contributors**
- **Common mtDNA types**
- **Mitochondrial DNA is a single locus**
  - **Bases are not independent**
- **Sensitivity**
  - **Typically require minimum 20% minor component for detection by sequencing**
- **Sequencing chemistry is not quantitative**

# ***Mitochondrial DNA Mixtures***

## ***Approaches to mtDNA Mixtures***

- **Sequencing**
- **Denaturing high-performance liquid chromatography (DHPLC)**
  - **Elution and collection of homo- and heteroduplex fractions**
- **Pyrosequencing**
  - **Linear relationship between incorporated nucleotides and amount of released light**
- **Mass spectrometry**

# ***Mitochondrial DNA Mixtures***

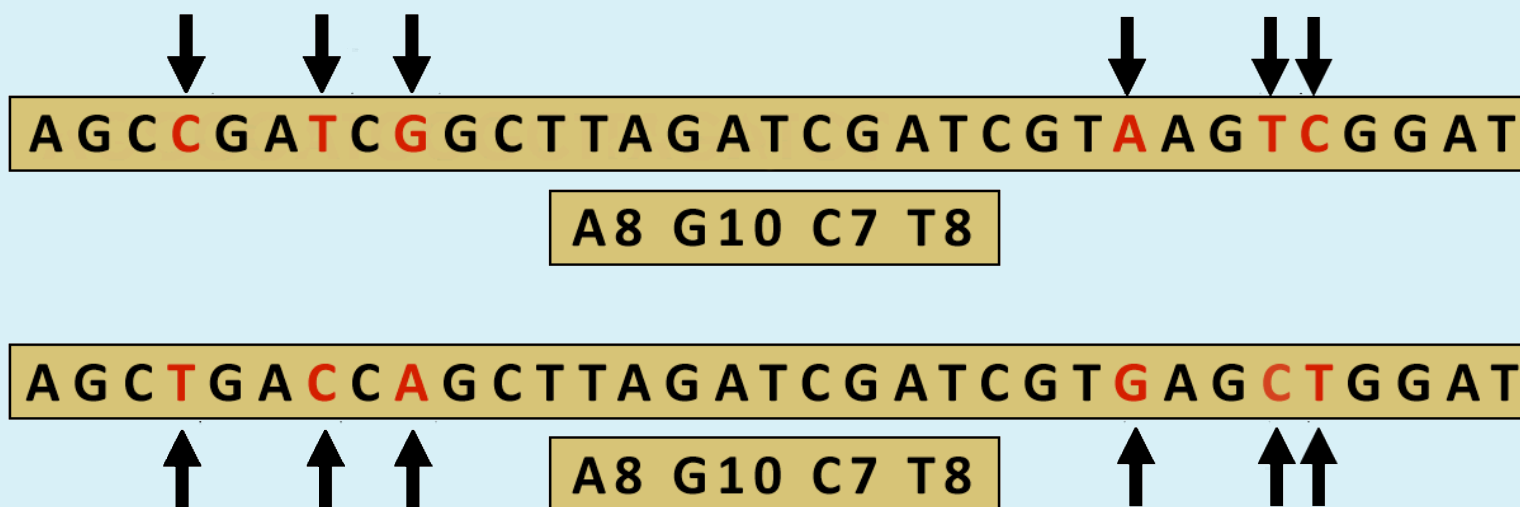
## ***Mass Spectrometry***

- **Ionized fragments are detected independently**
- **Multiple mtDNA types will generate multiple signals**
- **Signal intensities reflect relative amounts within mixed sample**
- **Quantitation and resolution of components**
- **Components must possess different molecular masses to be distinguished**
  - **Compensatory changes are undetectable**



# Mitochondrial DNA Mixtures

## Compensatory Changes



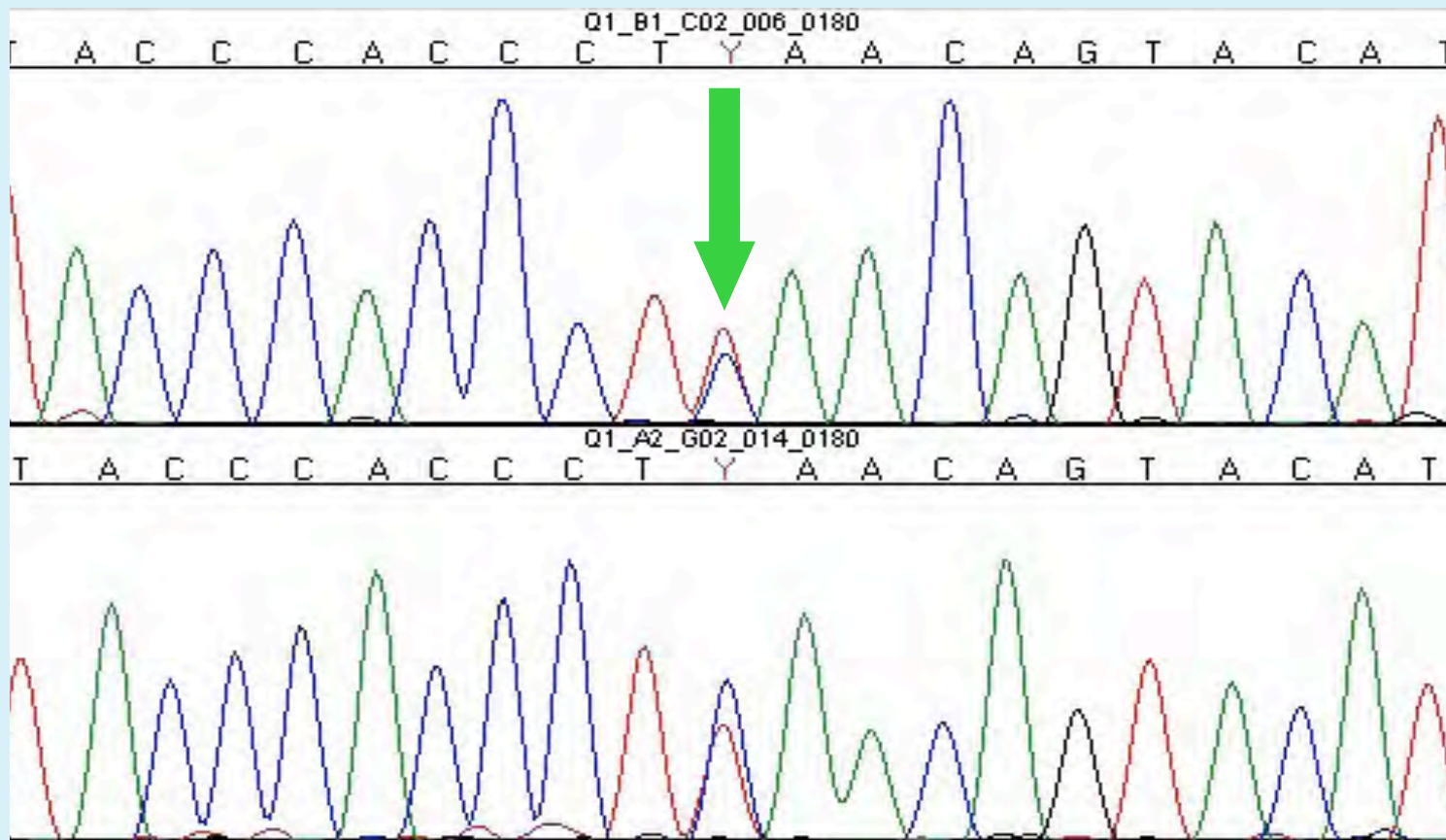
Graphic courtesy of Leslie D. McCurdy, Ph.D.

# ***Mitochondrial DNA Mixtures***

## ***Natural mtDNA Mixtures***

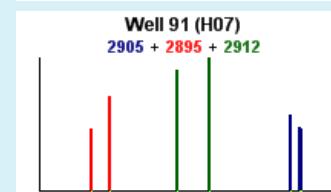
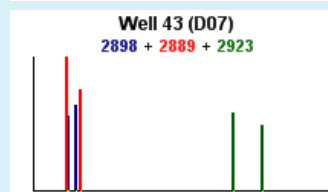
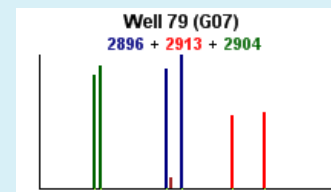
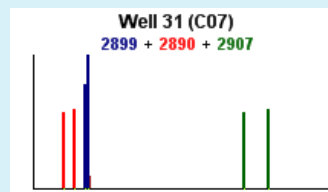
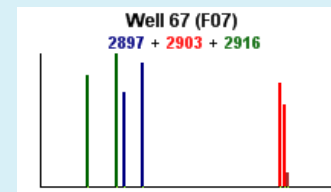
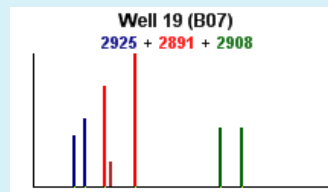
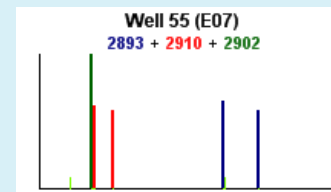
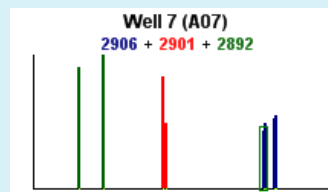
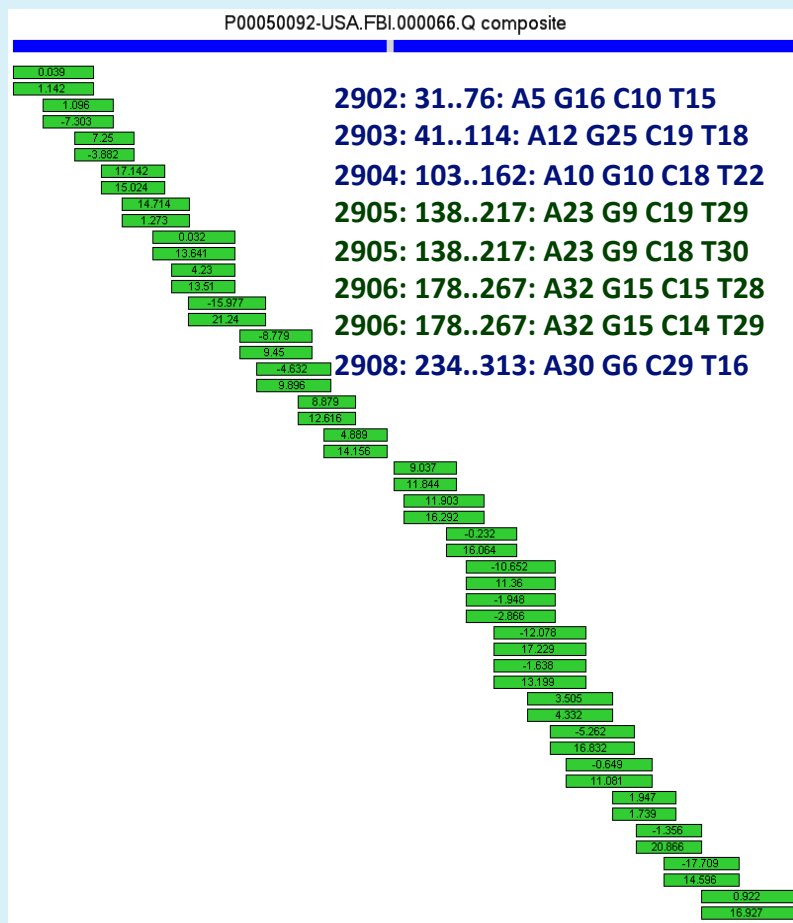
- **Heteroplasmy**
- **Use known heteroplasmic mtDNA types**
  - Point
  - Length
    - HV1, HV2, HV3
- **Perform Ibis™ mtDNA Assay**
- **Observe sensitivity and reproducibility**
  - Tissue types and within tissue/sample

# Mitochondrial Point Heteroplasmy



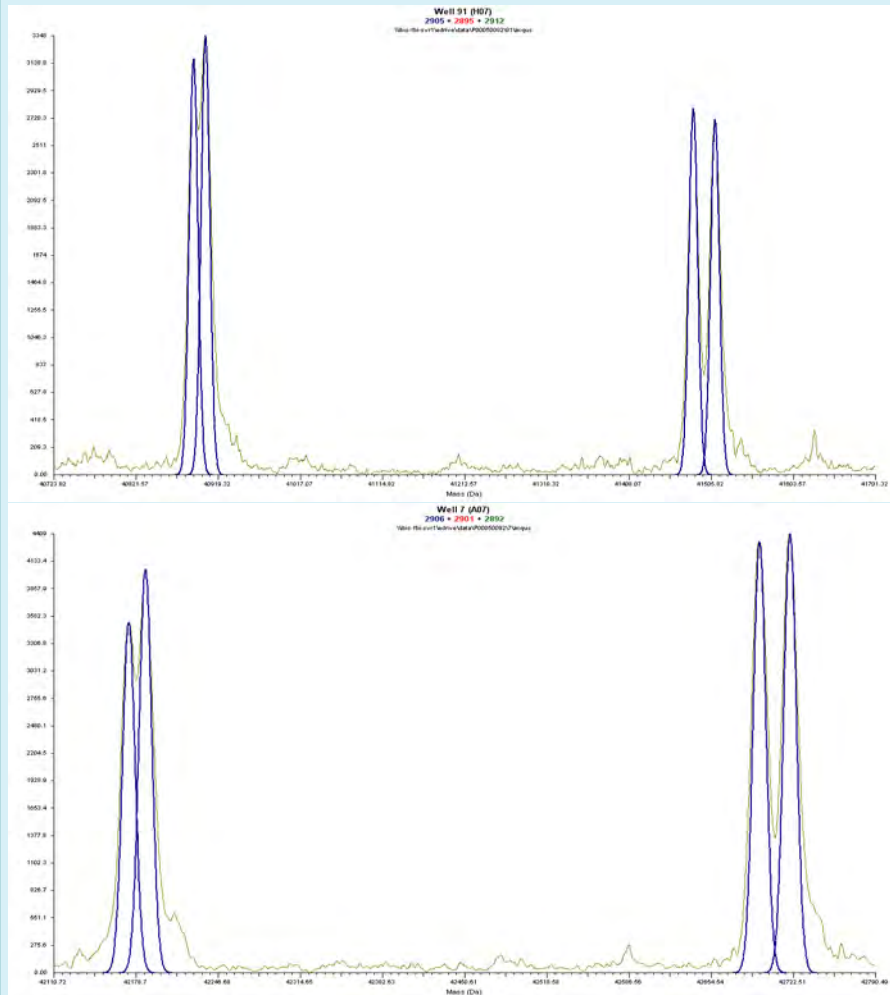
Images courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA Point Heteroplasmy at 195 Y



Images courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA Point Heteroplasmy at 195 Y



2905: 138..217: A23 G9 C19 T29

2905: 138..217: A23 G9 C18 T30

Well 91 49%

51%

2906: 178..267: A32 G15 C15 T28

2906: 178..267: A32 G15 C14 T29

Well 7 49%

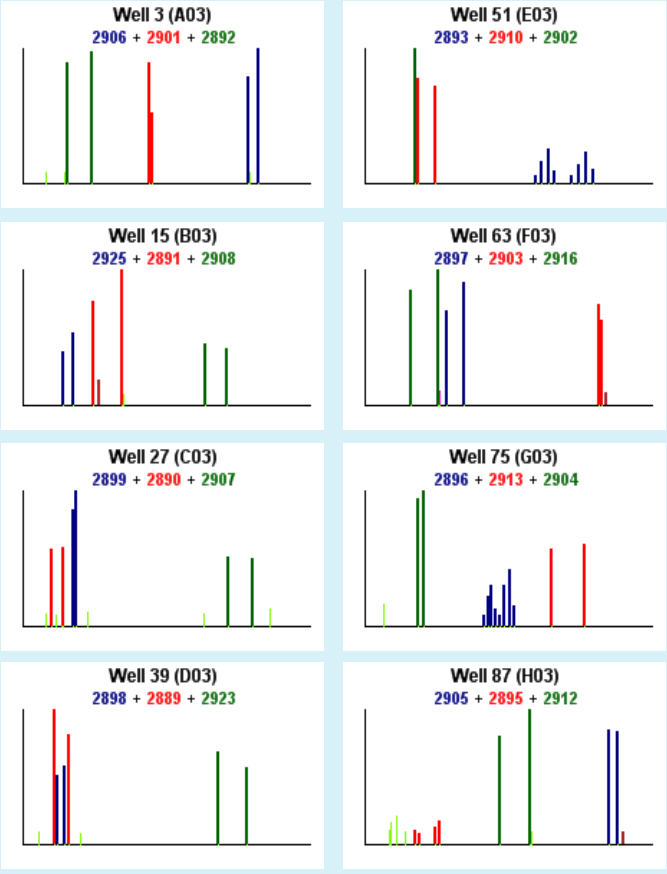
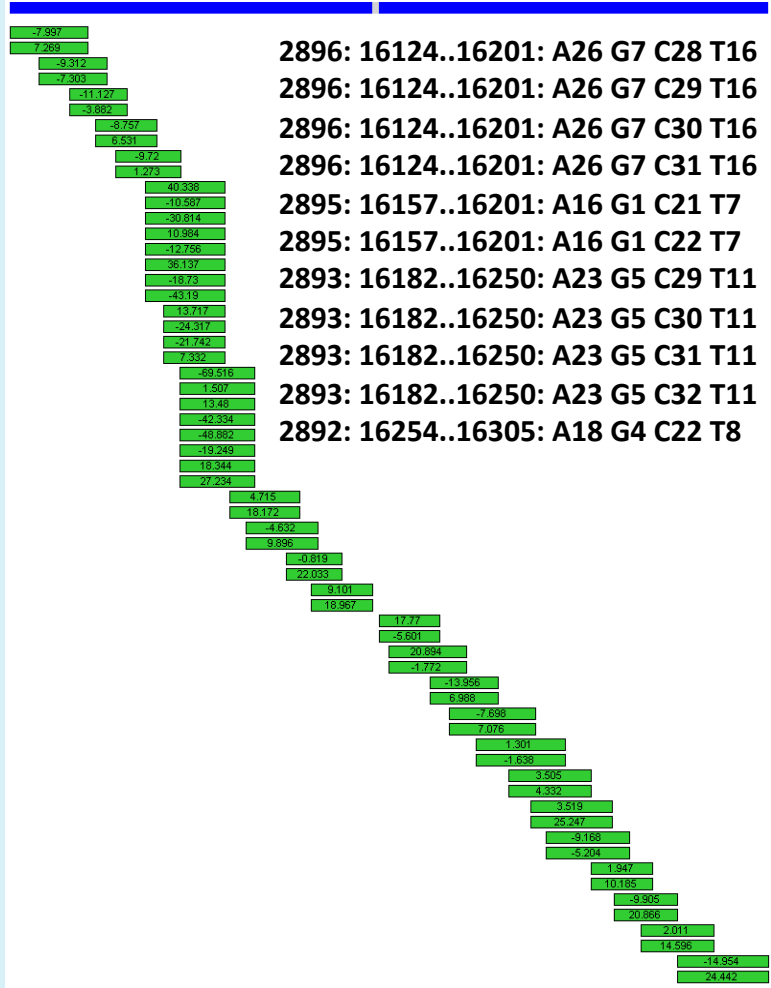
51%

Images courtesy of Leslie D. McCurdy, Ph.D.



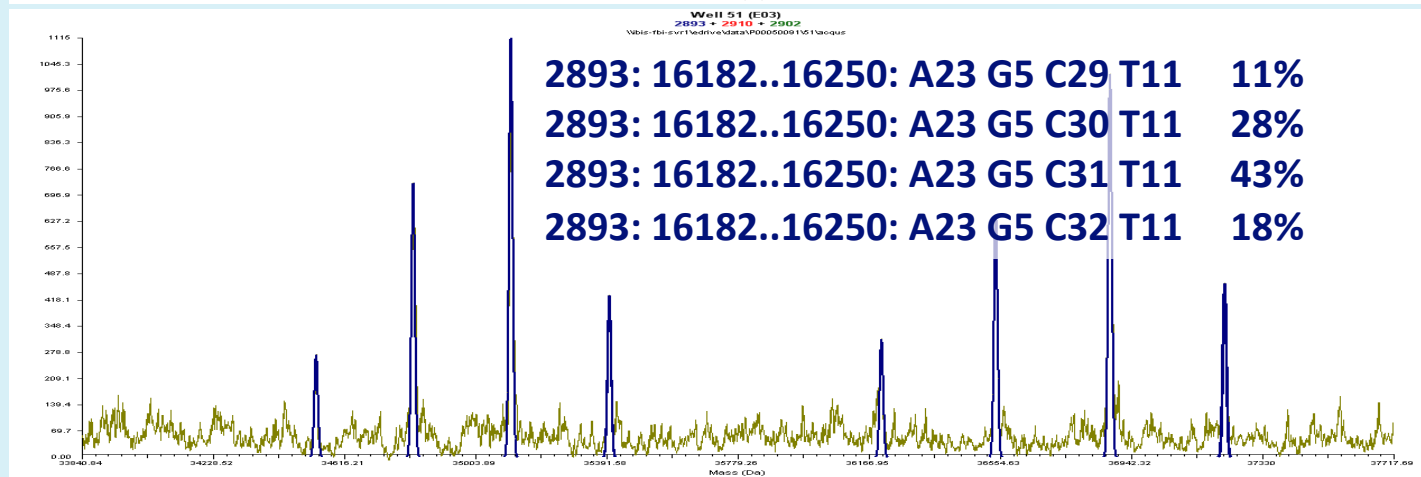
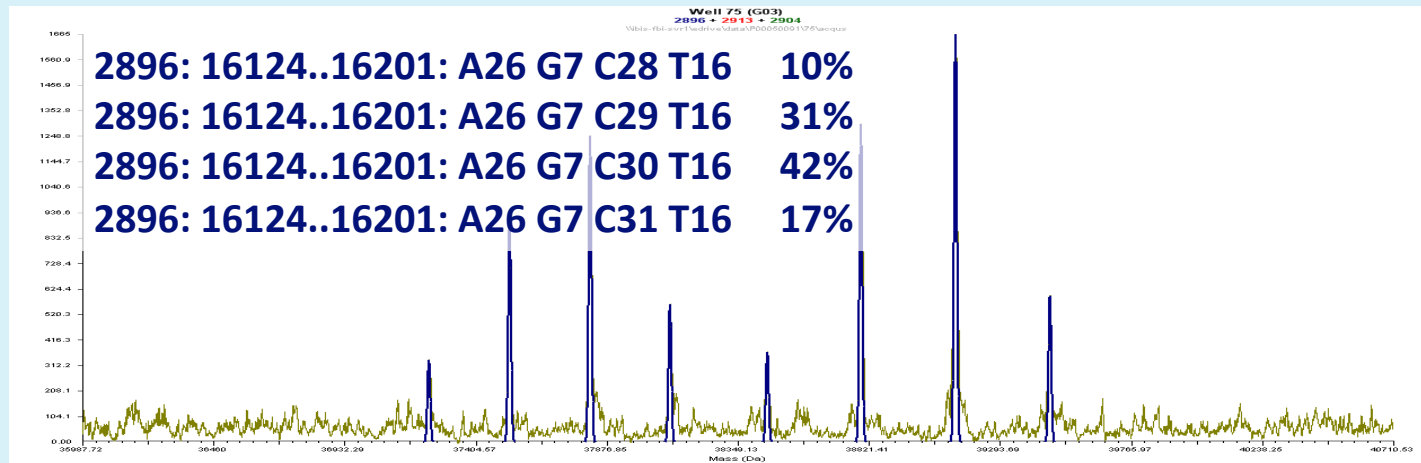
# mtDNA HV1 Length Heteroplasmy

P00050091-USA.FBI.000009.Q composite



Images courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA HV1 Length Heteroplasmy



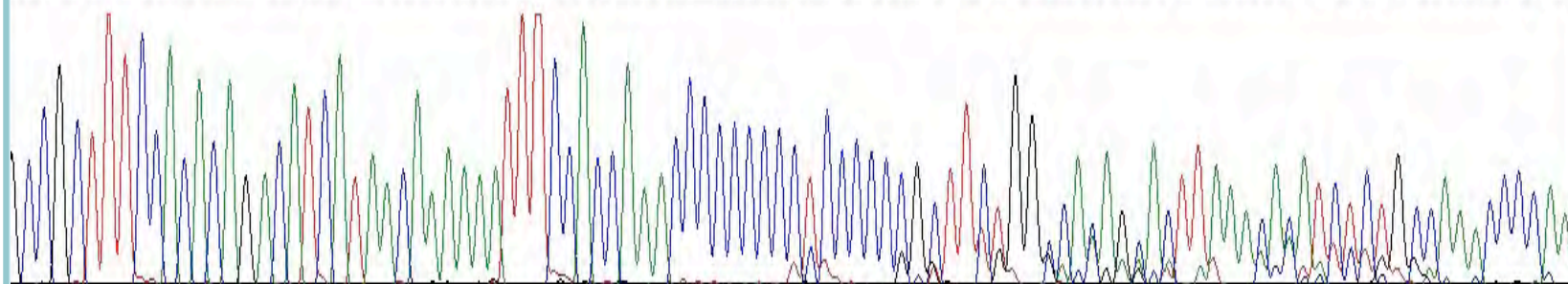
Images courtesy of Leslie D. McCurdy, Ph.D.



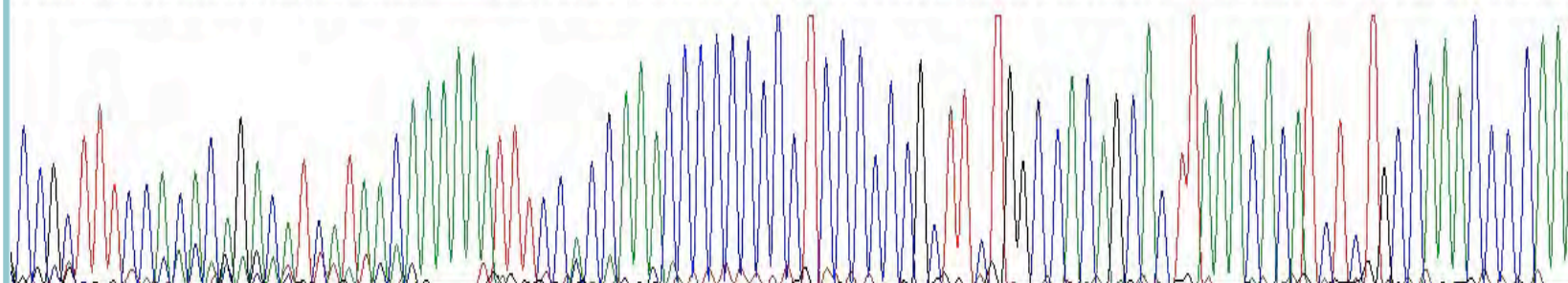
# mtDNA HV2 Length Heteroplasmy

*Mixed sequence after length variation*

K4\_C1\_G02\_006\_0443 Fragment base #309.2. Base 263 of 436  
G CCG CTTTCCACACAG ACATCAT AACAAAAAATTTCCACCA A ACCCCCCCCT CCCCCCG CTTCTGG CCANAGCACTTAAACACATCTCTGCCAAACCCCAA



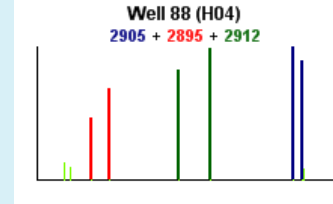
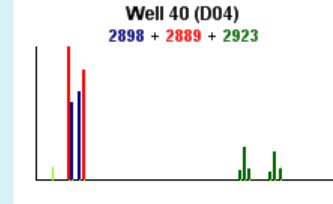
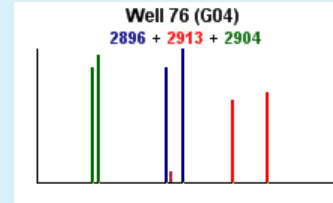
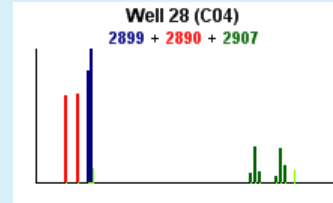
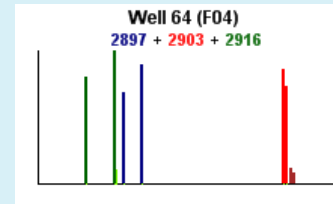
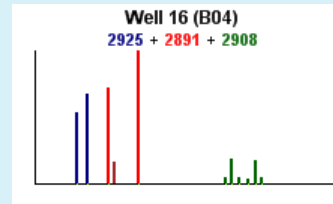
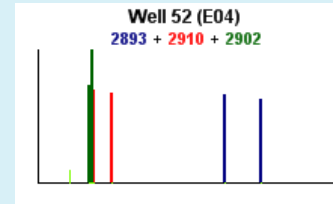
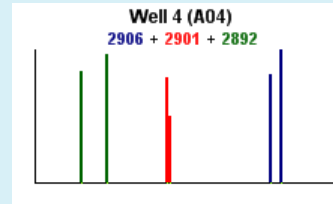
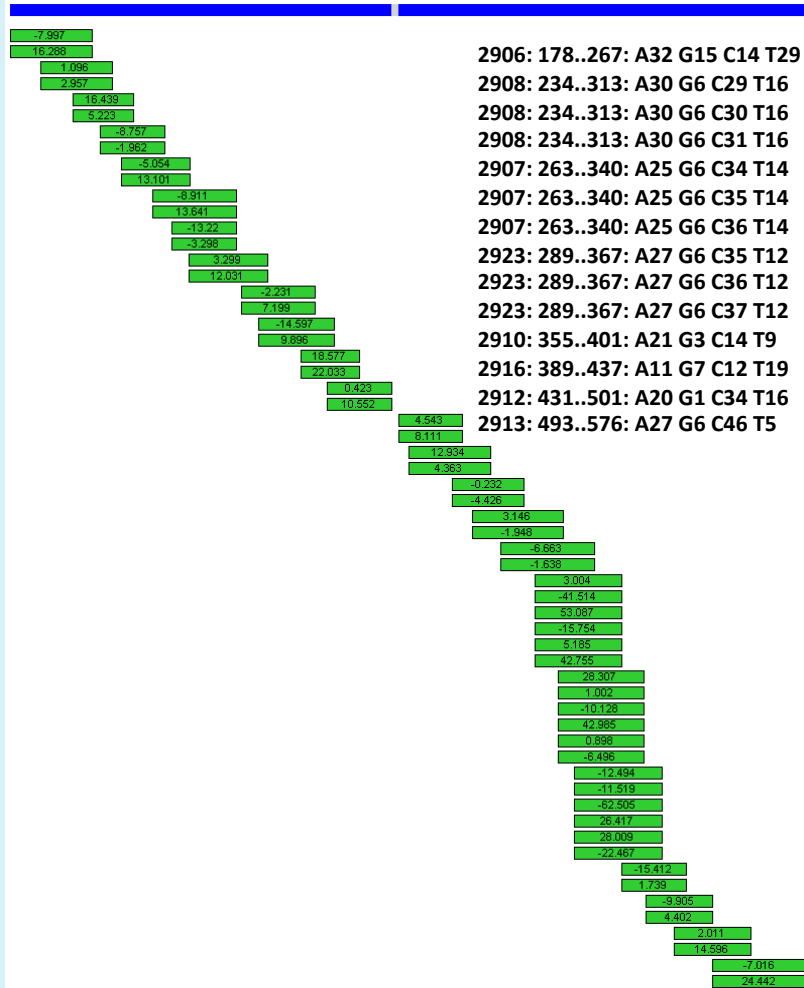
K4\_D1\_G02\_014\_0443 Fragment base #309.2. Base 263 of 363  
G CCGCTTTC CACACAGACATCATAACAAAAAATTTCCACCA A ACCCCCCCCT CCCCCCGCTTCTGGC CACAGCAC TTAAACACATCTCTGCCAAACCCCAA



Images courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA HV2 Length Heteroplasmy

P00050091-USA.FBI.000022.Q composite



Images courtesy of Leslie D. McCurdy, Ph.D.

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# mtDNA HV2 Length Heteroplasmy

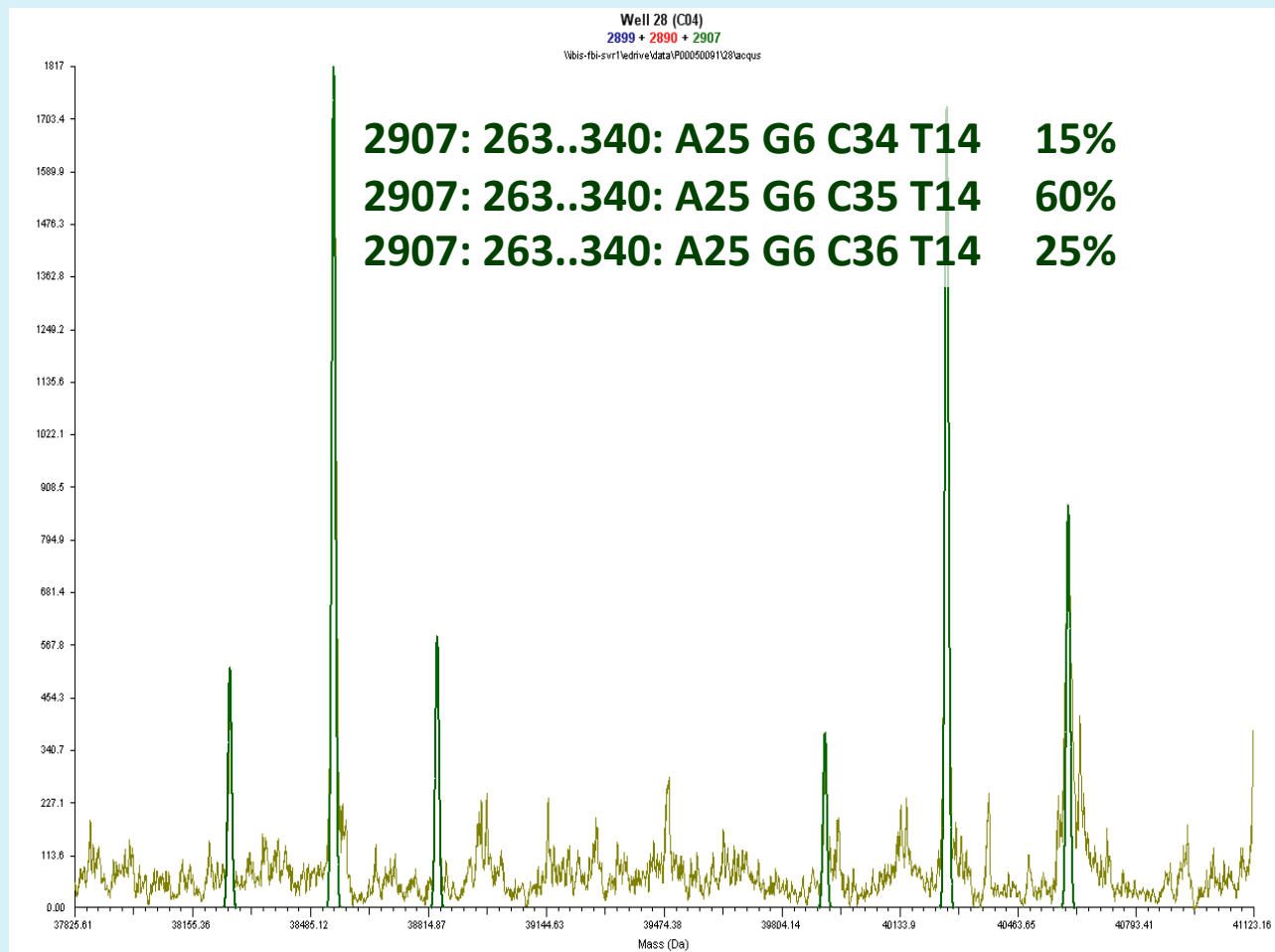
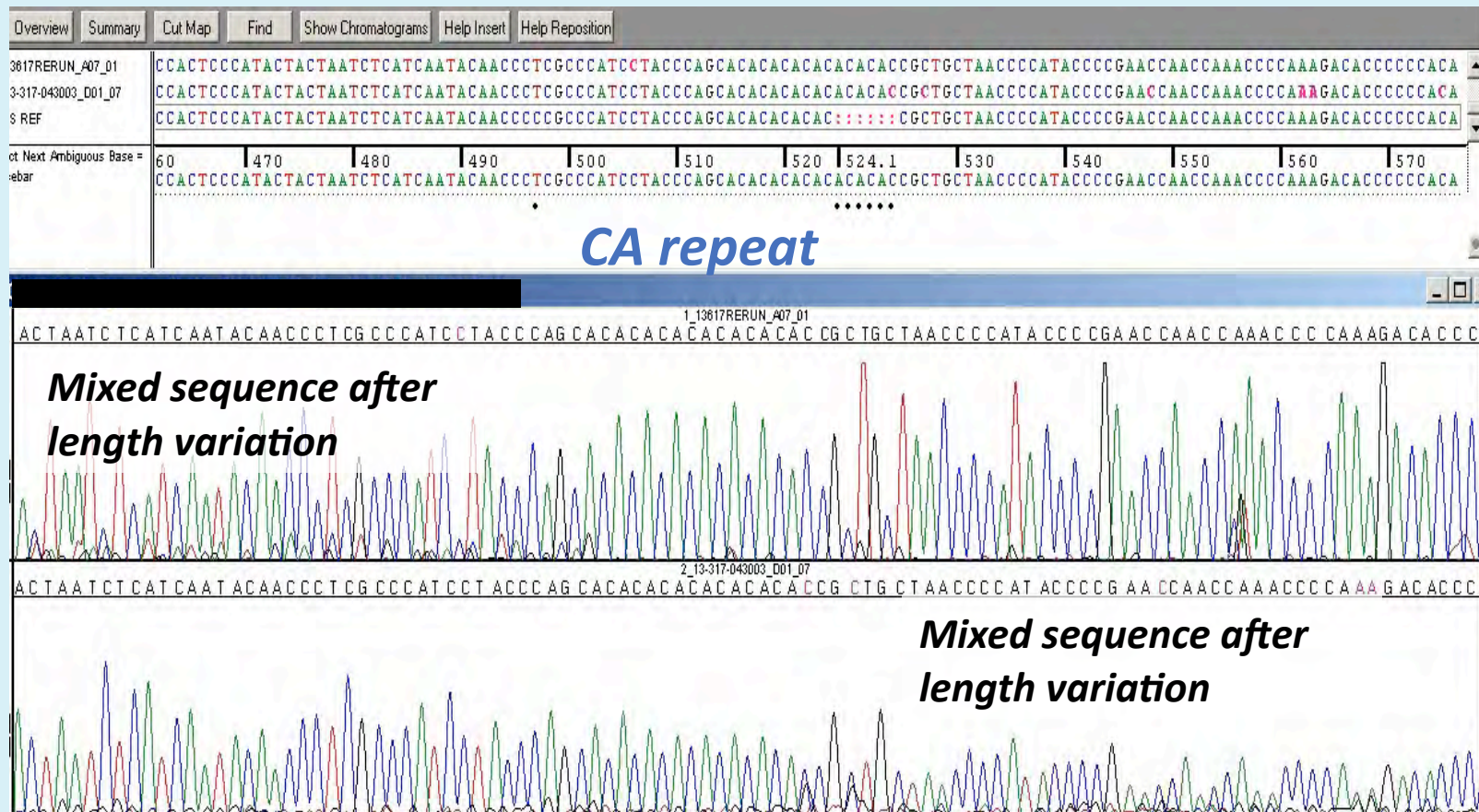


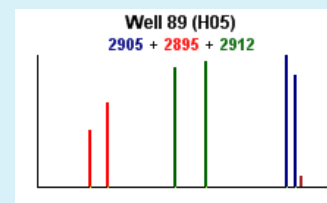
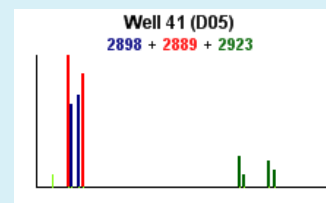
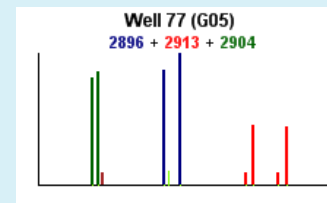
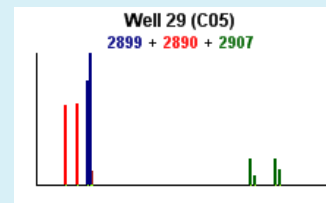
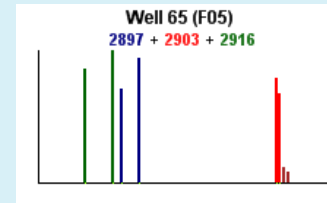
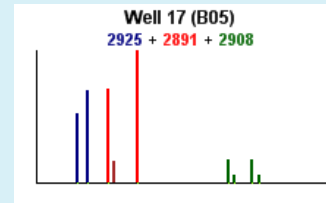
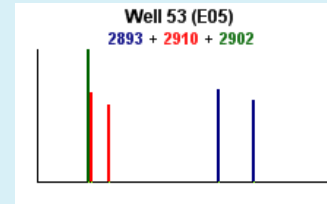
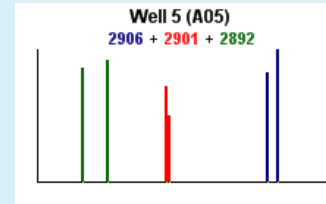
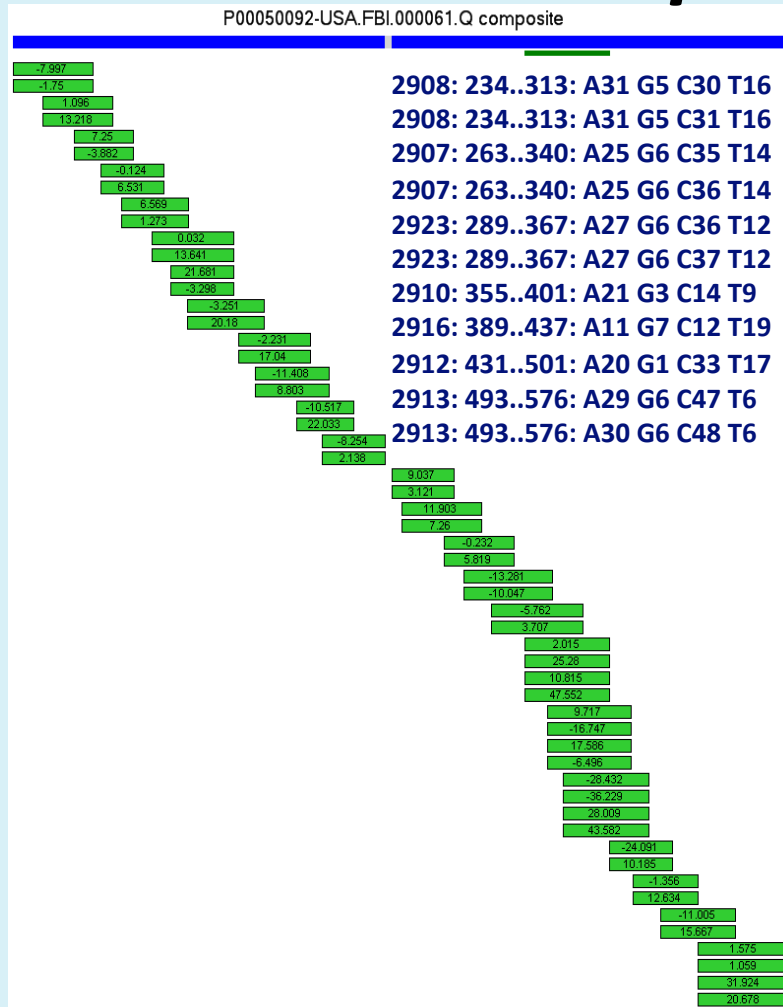
Image courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA HV3 CA Repeat Heteroplasmy



Images courtesy of Leslie D. McCurdy, Ph.D.

# mtDNA HV3 CA Repeat Heteroplasmy



Images courtesy of Leslie D. McCurdy, Ph.D.

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# mtDNA HV3 CA Repeat Heteroplasmy

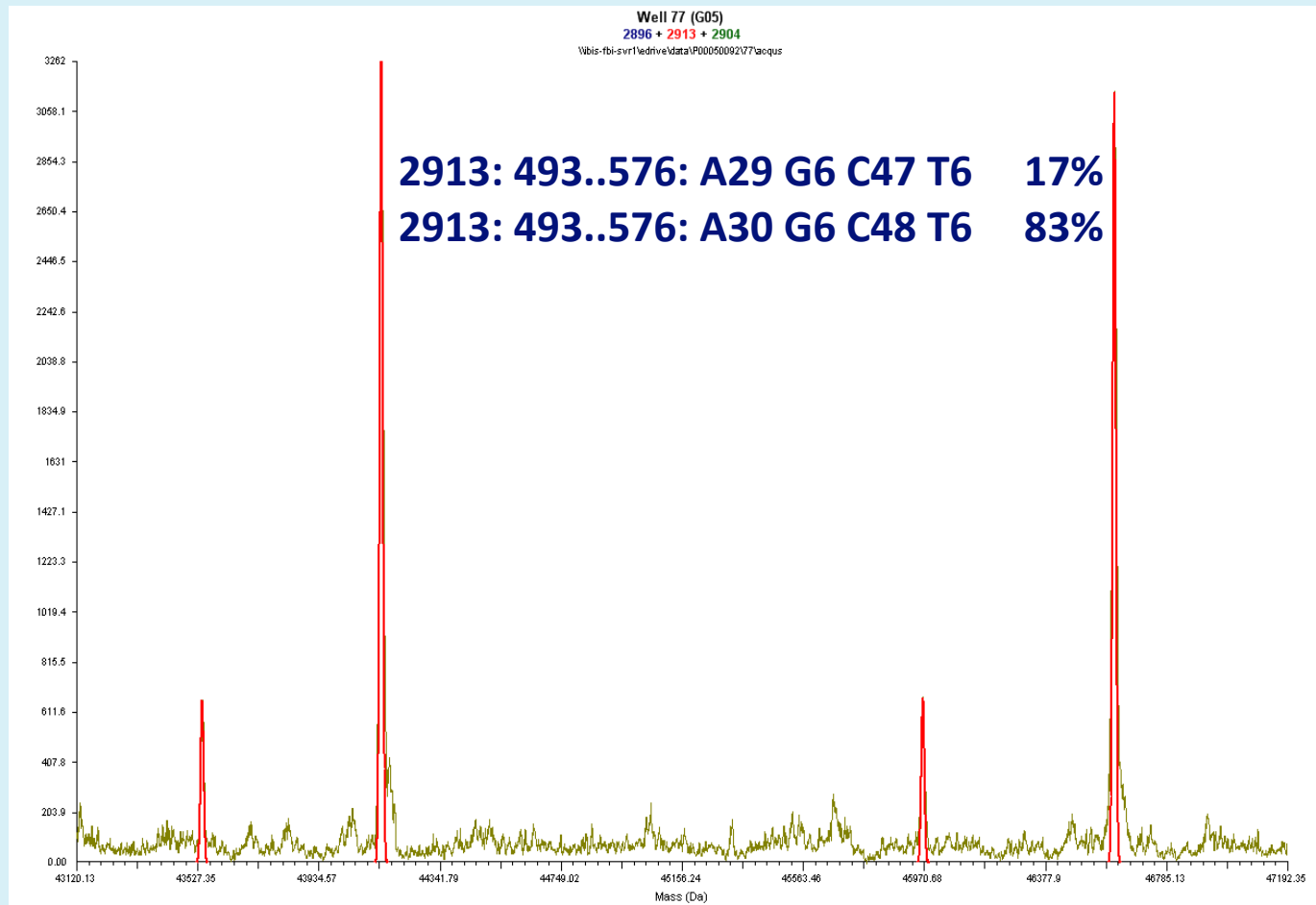


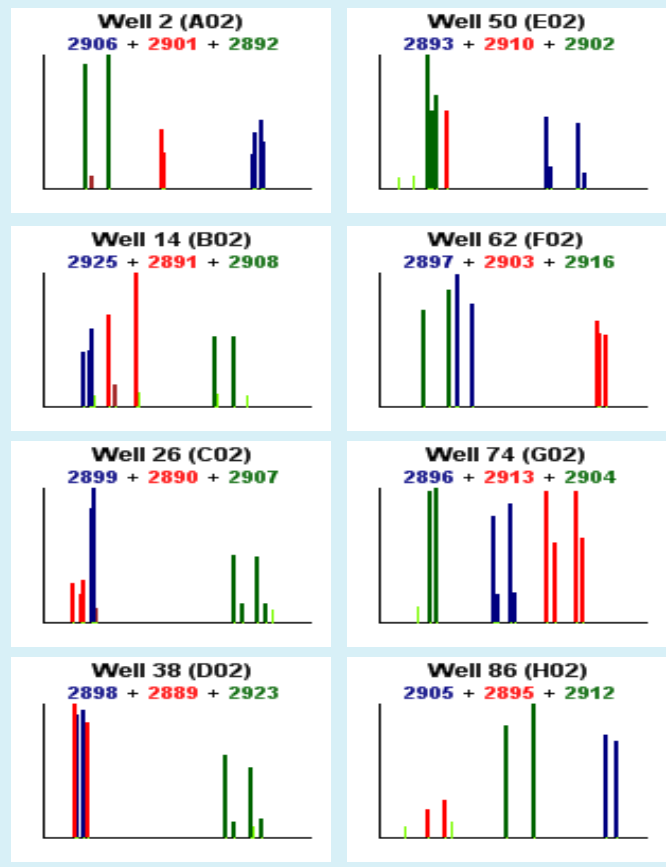
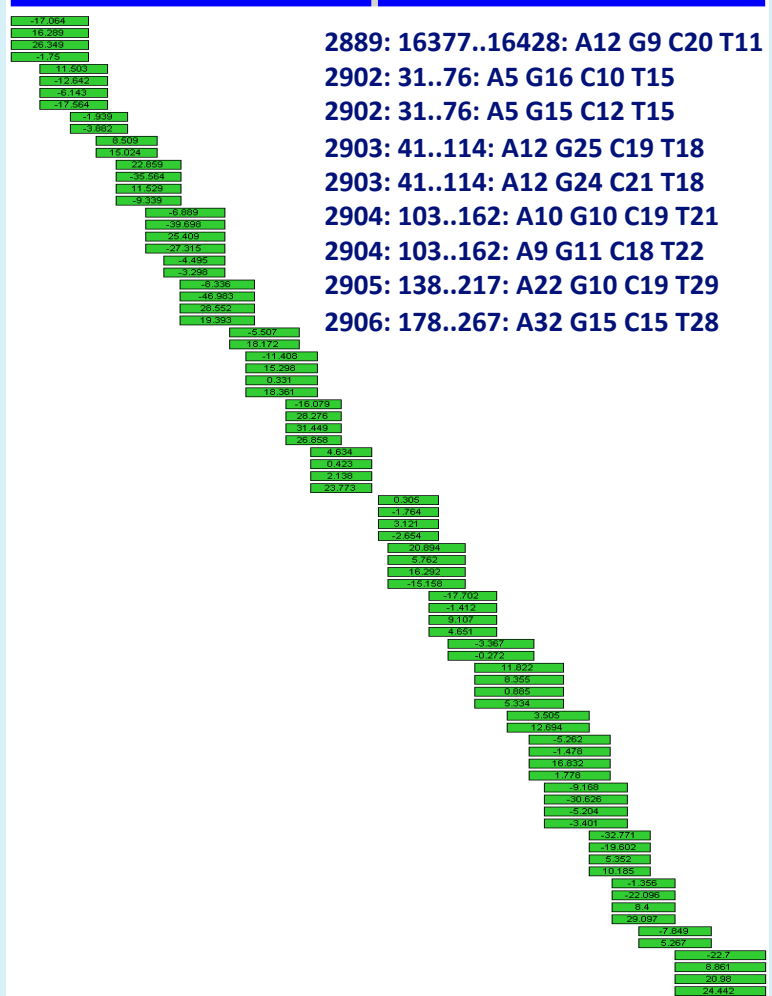
Image courtesy of Leslie D. McCurdy, Ph.D.

## ***Engineered mtDNA Mixtures***

- **Known mtDNA types extracted**
- **mtDNA quantified as copies/mL**
- **mtDNA combined at predetermined ratios:**
  - **50/50**
  - **75/25**
  - **90/10**
- **Ibis™ mtDNA Assay was performed**

# 50:50 Ratio Mixture of mtDNA

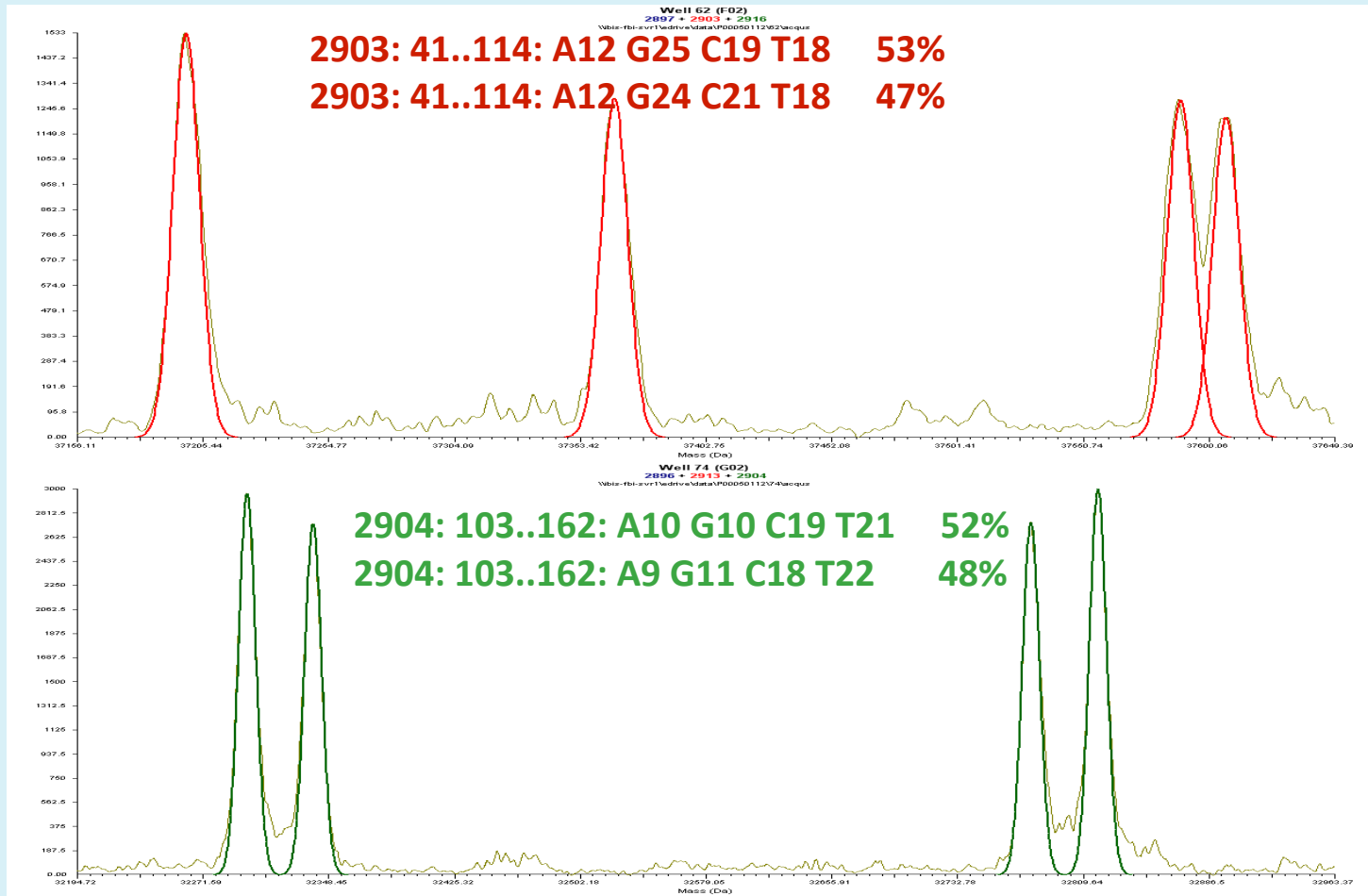
P00050112-USA.FBI.000065\_USA.FBI.000070.BUCCAL\_composite



Images courtesy of Leslie D. McCurdy, Ph.D.

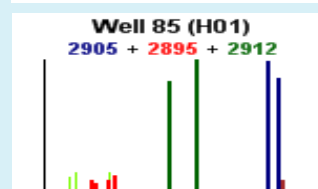
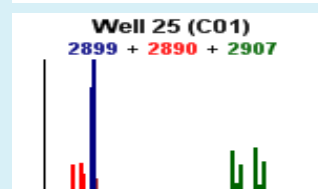
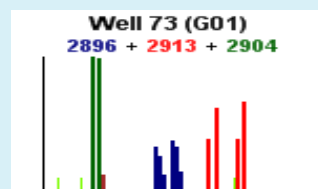
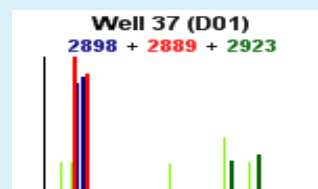
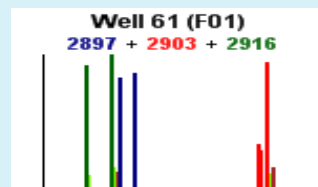
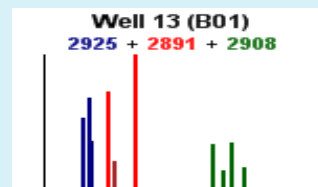
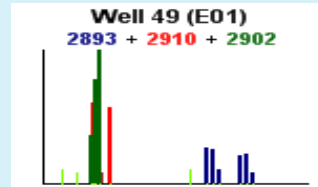
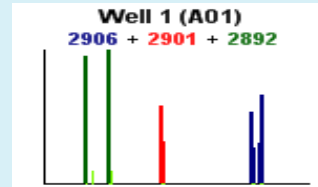
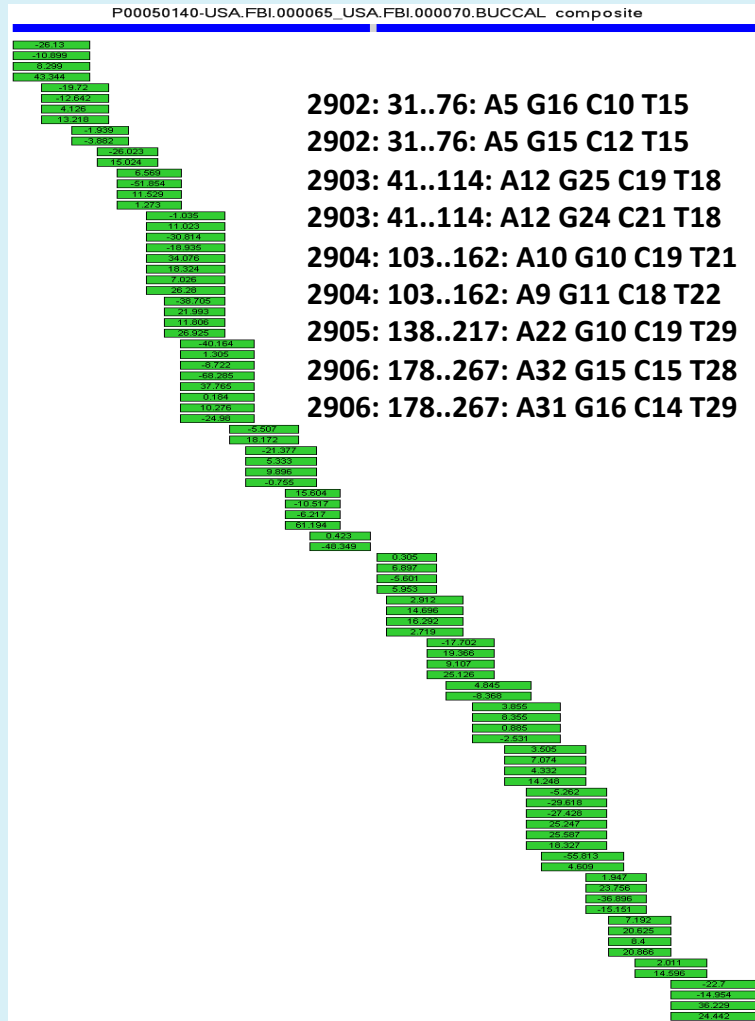


# 50:50 Ratio Mixture of mtDNA



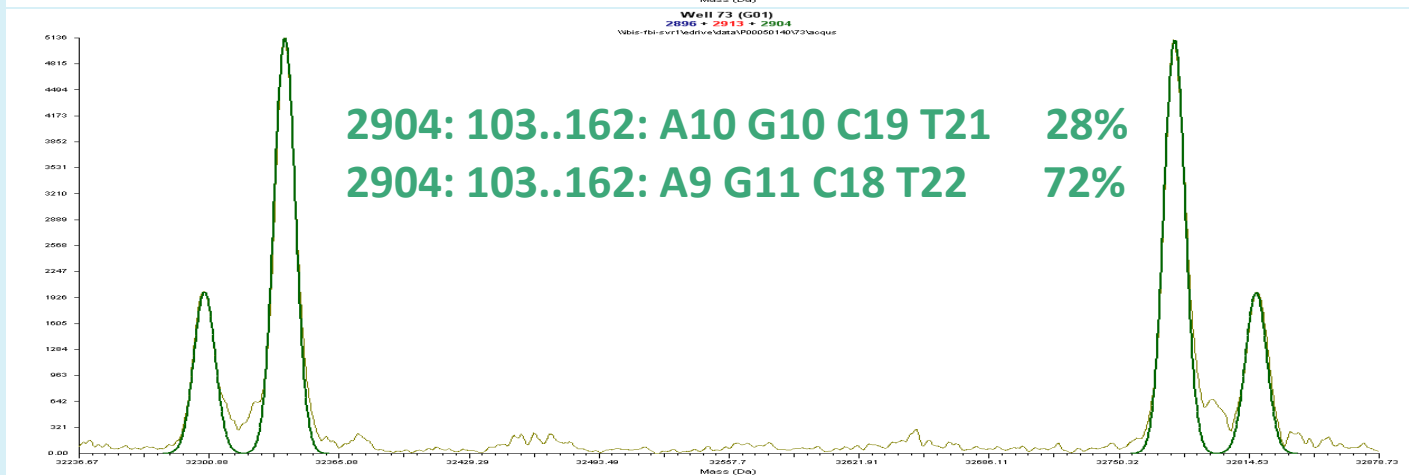
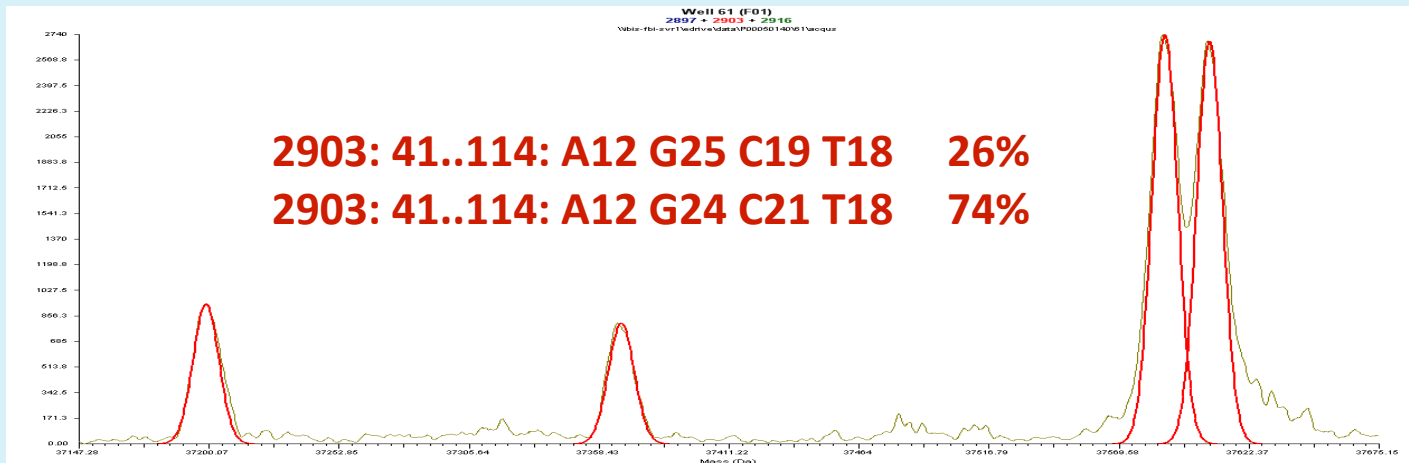
Images courtesy of Leslie D. McCurdy, Ph.D.

# 75:25 Ratio Mixture of mtDNA



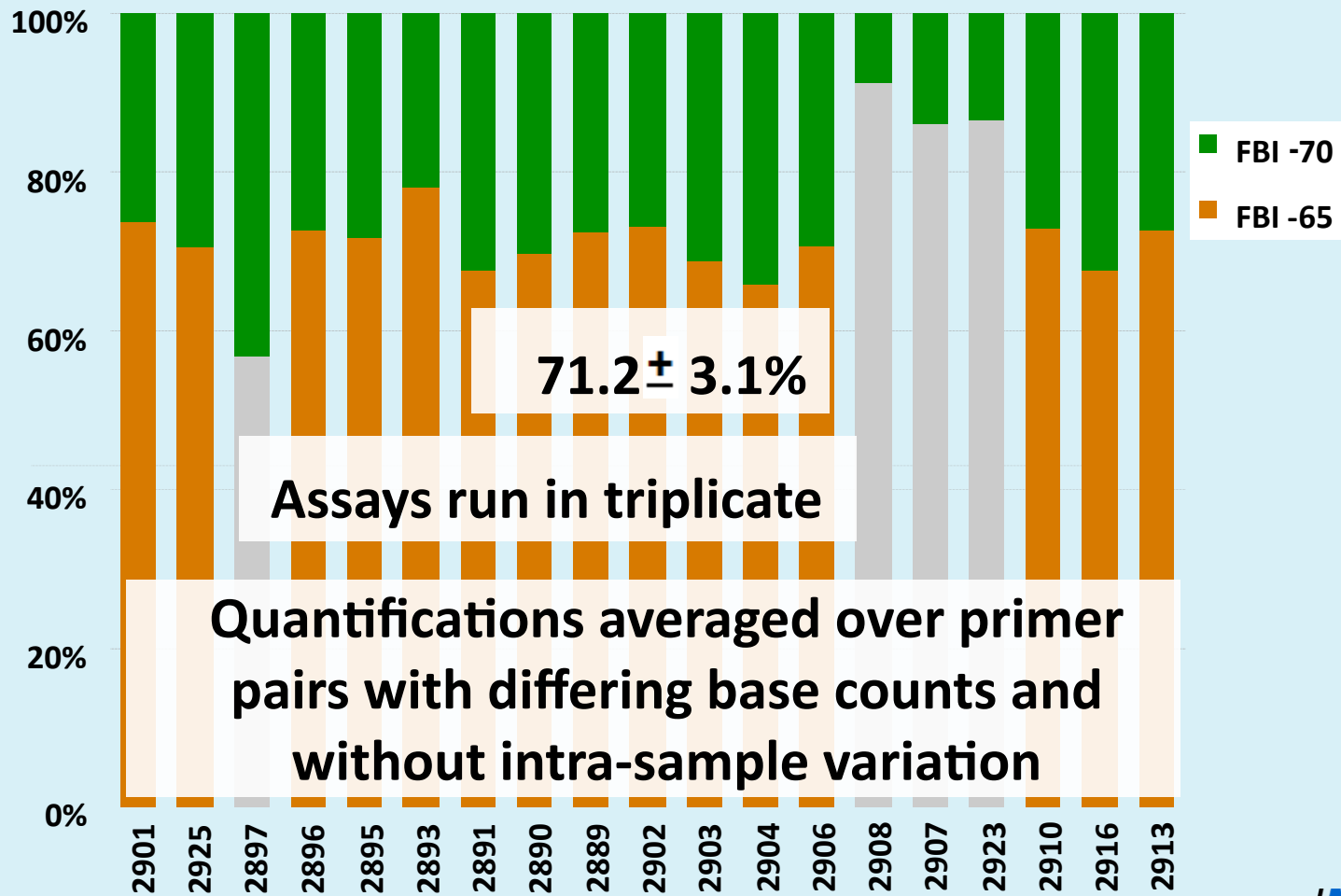
Images courtesy of Leslie D. McCurdy, Ph.D.

# 75:25 Ratio Mixture of mtDNA



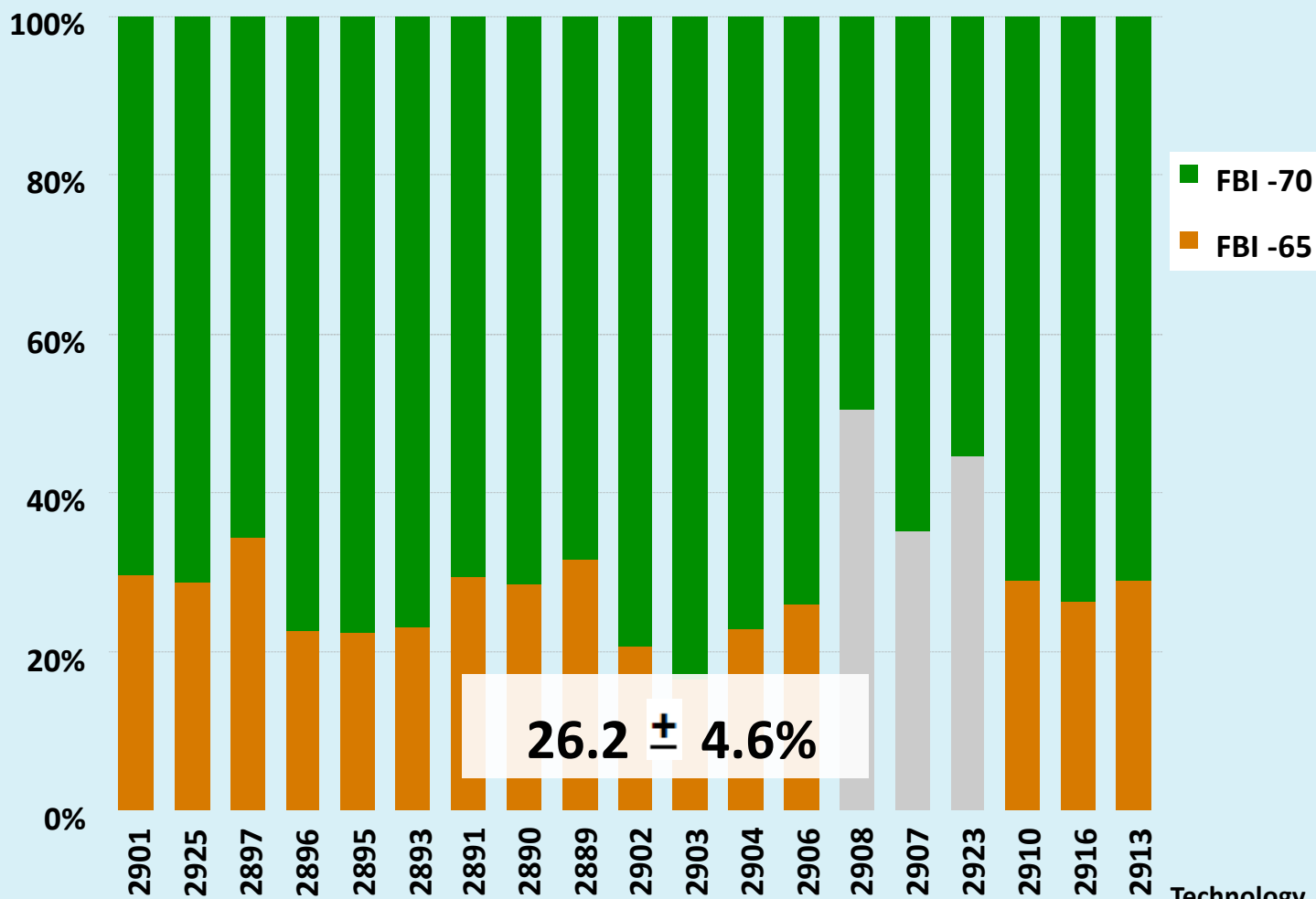
Images courtesy of Leslie D. McCurdy, Ph.D.

# 75:25 Ratio Mixture of mtDNA



Graphic courtesy of Thomas A. Hall, Ph.D.

# 25:75 Ratio Mixture of mtDNA



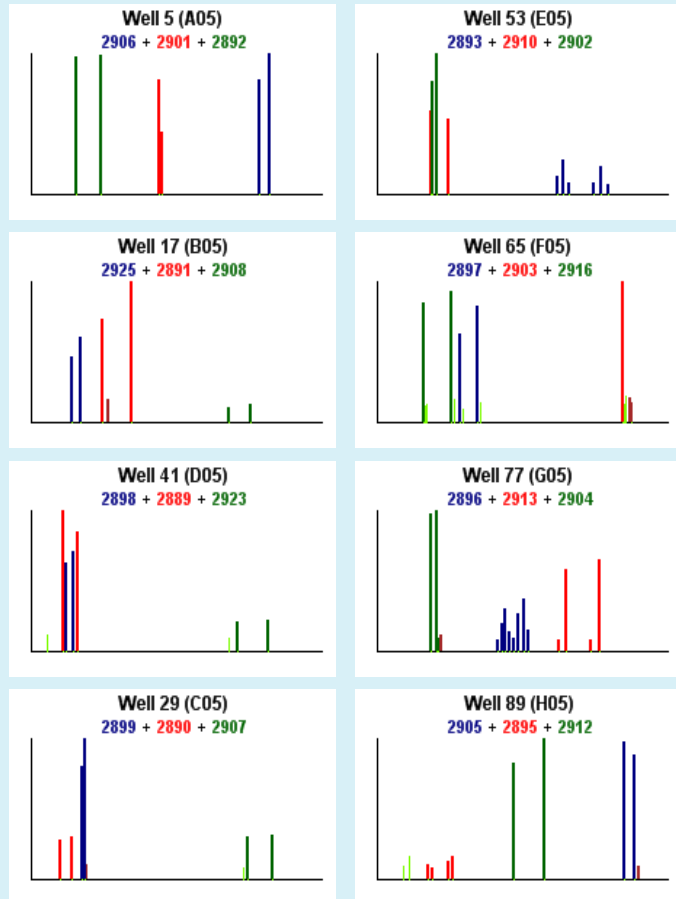
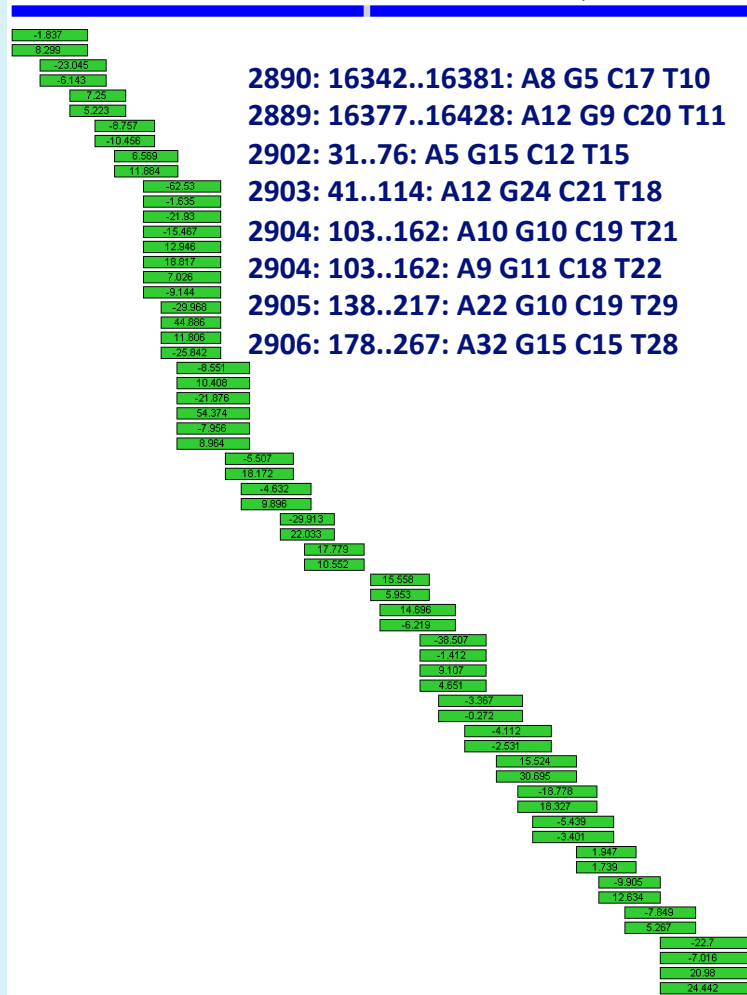
Graphic courtesy of Thomas A. Hall, Ph.D.

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# 90:10 Ratio Mixture of mtDNA

P00050140-USA.FBI.000065\_USA.FBI.000070.BUCCAL composite



Images courtesy of Leslie D. McCurdy, Ph.D.

# 90:10 Ratio Mixture of mtDNA

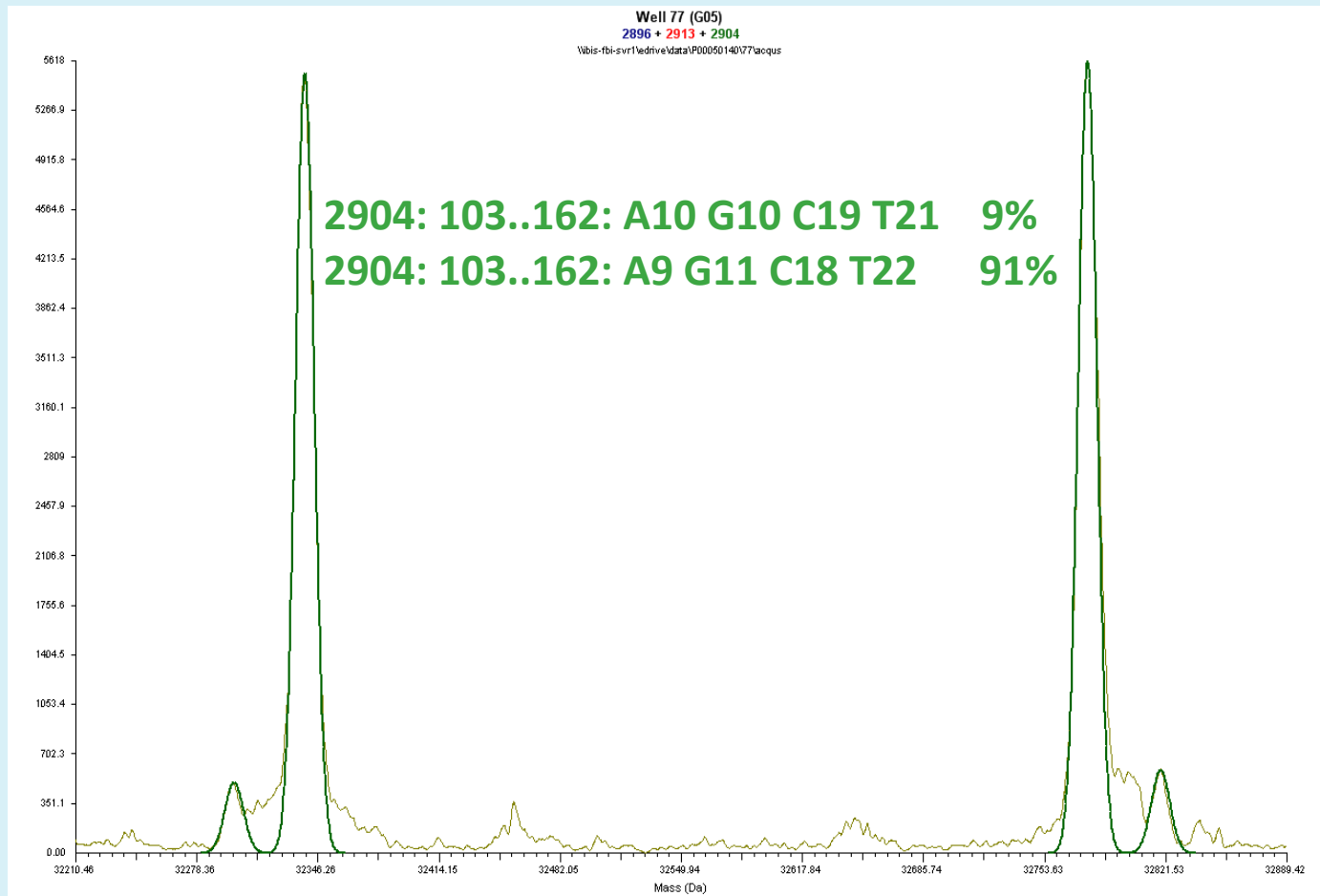
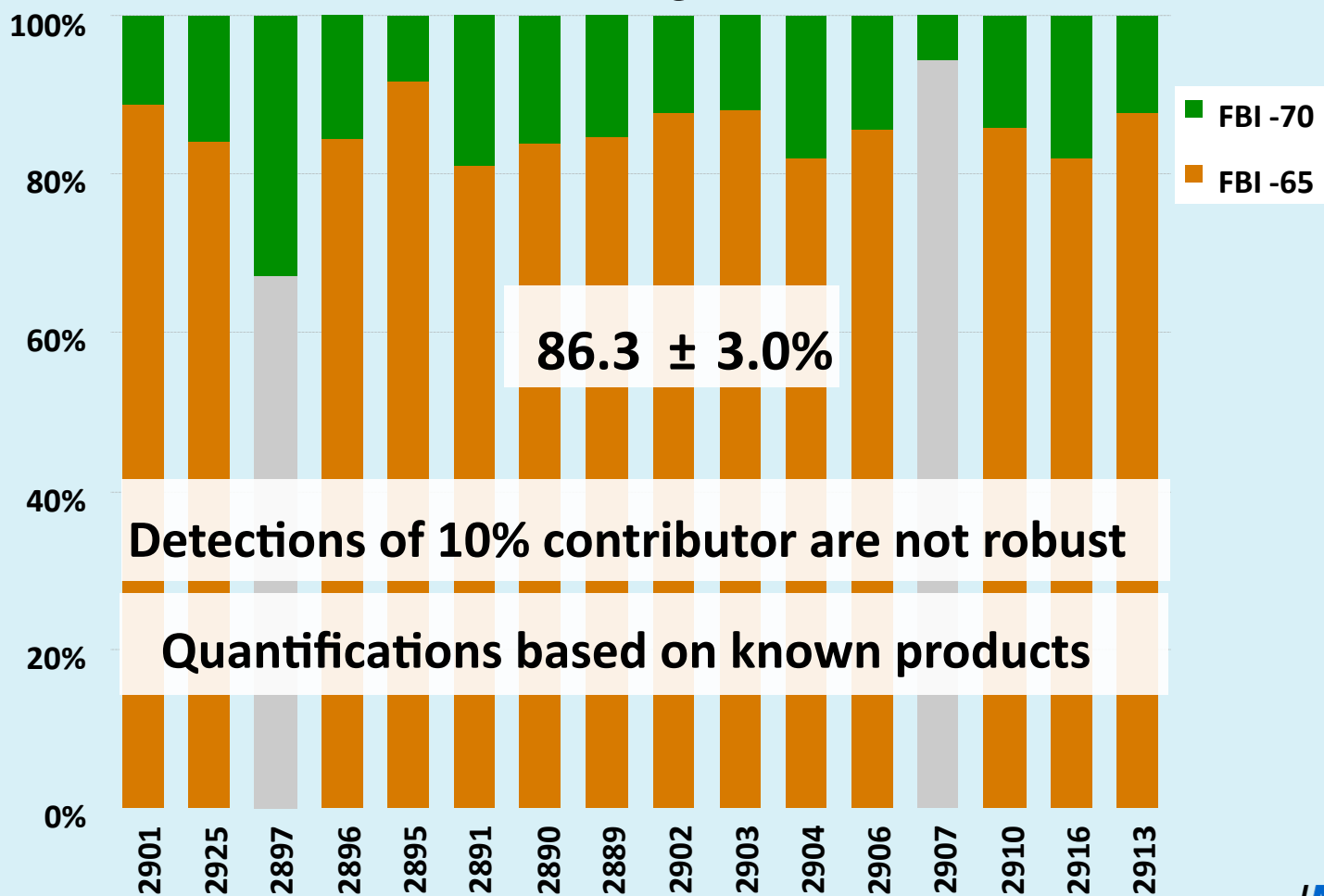


Image courtesy of Leslie D. McCurdy, Ph.D.

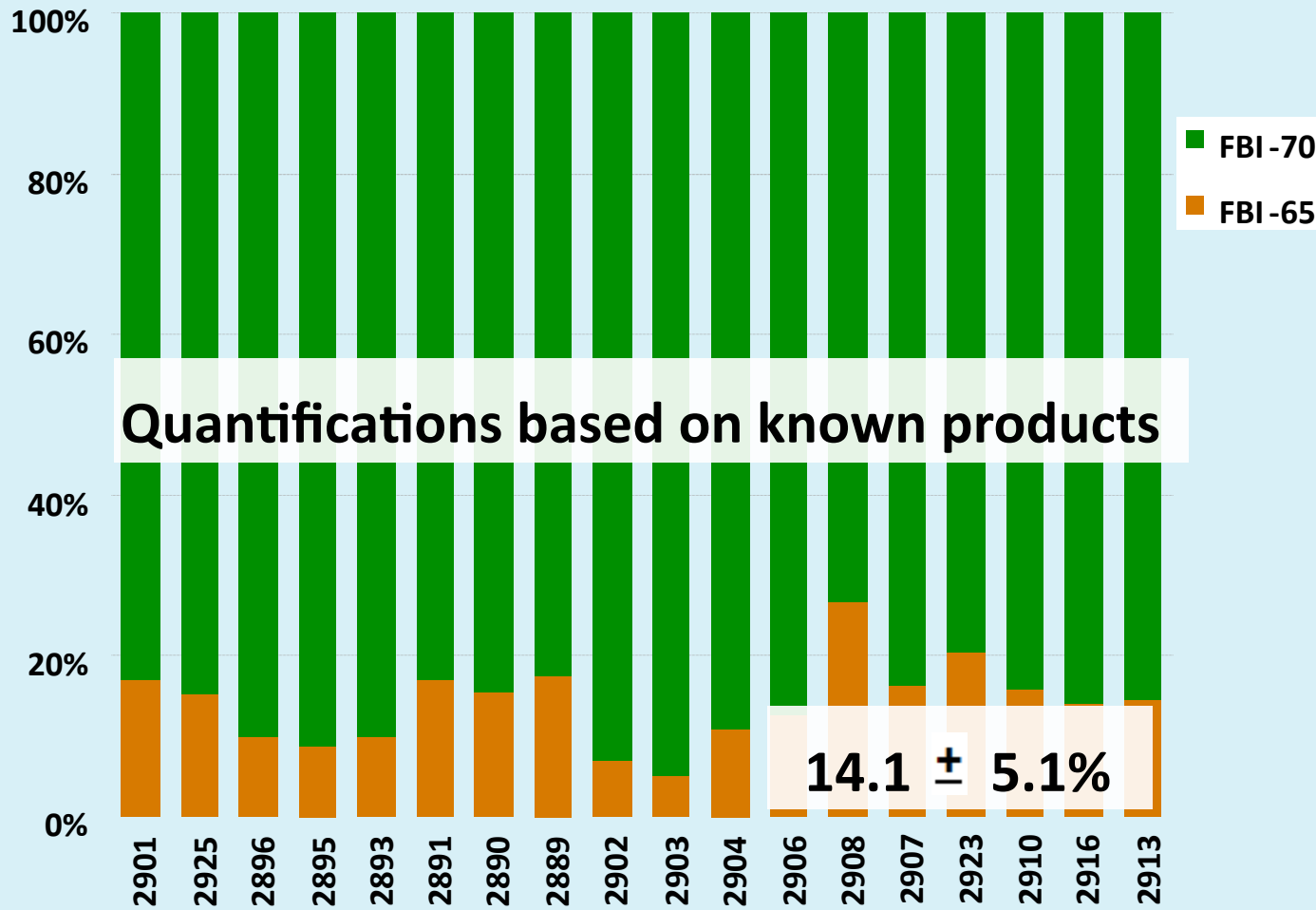
# 90:10 Ratio Mixture of mtDNA



Graphic courtesy of Thomas A. Hall, Ph.D.



# 10:90 Ratio Mixture of mtDNA



Graphic courtesy of Thomas A. Hall, Ph.D.

# ***Interpretation of mtDNA Mixtures***

## **Heteroplasmy**

- **Point heteroplasmy**
  - Concordant base composition profiles
  - Cannot exclude
- **Length heteroplasmy**
  - Absence of common base composition profiles should not be used for exclusionary purposes
  - Ignore differences due to indels within pp 2896, 2895, 2893 (HV1), 2908, 2907, 2923 (HV2), 2913 (HV3)

## **Two-contributor mixtures**

- **Subtract out major/minor components**
- **Reconstruct mtDNA profiles based on relative abundances**
- **Perform comparisons and database searches as appropriate**

# ***Caveats to mtDNA Mixture Interpretation***

- **Heteroplasmy in one or both contributors to a mixture can cause ambiguity in profile deconvolution**
  - **Heteroplasmic products at a locus should be considered possible for both profiles**
  - **Products divergent from ratios observed for other products may represent common base count with heteroplasmy in one individual and should be considered ambiguous**
- **Multiple contributors should be considered non-interpretable**
  - **No attempt to deconvolve profiles for 3+ contributors**

# ***STR Mixtures***

- **AB Identifiler® kit reported robust at 1:10 template input ratios and detectable at 1:20 ratios**
- **Ibis™ STR Assay currently not able to detect 1:10 mixtures**
  - Continuing development – current data is preliminary
  - Mixture detection requires ~1:5 ratio
  - Profile deconvolution requires a known profile hypothesis
  - Limitation is biochemical noise baseline and intra-locus balance of multiplexed PCR output, not quantitative ability of instrument
  - Quantification should be as good or better than CGE in future
- **Advantage of Ibis™ system for mixtures is ability to resolve mixed alleles of the same length that differ by a polymorphism**

# STR Mixtures

+

LOCUS	55-24622	55-25578
AMEL	X, Y	X, Y
CSF1P0	10, 12	10, 13
D13S317	10, 12 (A → T)	8, 12 (A → T)
D16S539	11, 12	11, 13
D18S51	14, 15	11, 16
D21S11	28, 32.2	27, 28
D3S1358	13, 17	14 (G → A), 15 (G → A)
D5S818	9 (G → T), 11	11, 13
D7S820	11, 12	8, 10
D8S1179	9, 11	12 (A → G), 15 (A → G)
FGA	20, 25	19, 21
THO1	7, 10	6, 9
TPOX	8, 11	8, 11
vWA	14 (A → G + 2T → 2C), 15 (G → A)	18, 19

Table courtesy of Thomas A. Hall, Ph.D.

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# STR Mixtures

1:2.5 Ratio of 55-25578 to 55-24622 (two blood samples)

3 loci in reaction:

D16S539

THO1

D13S317

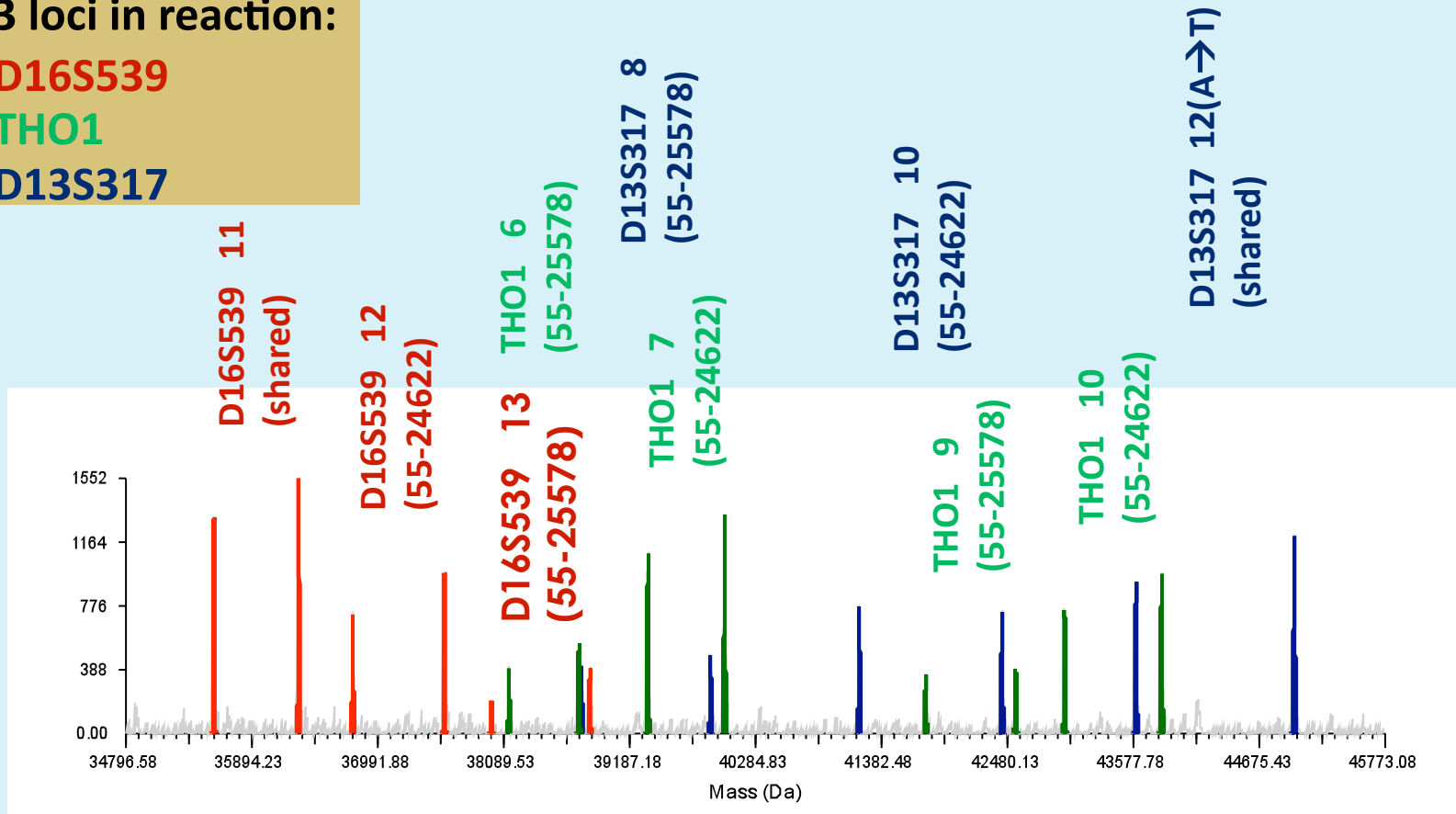


Image courtesy of Thomas A. Hall, Ph.D.

## ***STR Mixtures***

- DNA templates mixed together in defined ratios
- DNA quantities estimated by Quantifiler®
- Major contributor concentration set at 2 ng/reaction
- Output signals averaged over all primer pairs

<b>Input Ratio</b>	<b>Output Signal Ratio</b>
<b>1 : 2.5</b>	<b>1 : 2.54 ± 0.70</b>
<b>1 : 2</b>	<b>1 : 2.06 ± 0.52</b>
<b>1 : 1.5</b>	<b>1 : 1.58 ± 0.31</b>
<b>1 : 1</b>	<b>1 : 1.09 ± 0.3</b>
<b>1.5 : 1</b>	<b>1.50 ± 0.25 : 1</b>
<b>2 : 1</b>	<b>1.89 ± 0.47 : 1</b>
<b>2.5 : 1</b>	<b>2.22 ± 0.47 : 1</b>

Table courtesy of Thomas A. Hall, Ph.D.

# Summary

- **Ibis™ mtDNA Assay is reproducibly quantitative**
  - Length heteroplasmy has little negative impact on profile detection
    - Length heteroplasmy is quantifiable, but impacts deconvolution of mixed profiles at heteroplasmic products
  - Two-contributor profiles can be deconvolved
  - 10% minor component can be quantified
  - With 15% or higher minor component, two unknown profiles can be deconvolved and non-heteroplasmic products assigned
- **Ibis™ STR Assay is quantitative over narrow range**
  - Ratios beyond 2.5 are currently sub-optimal
  - Assay will improve in the future
  - Mixed alleles of the same length can be differentiated



***Questions?***

# ***Contact Information***

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