



Mitochondrial DNA Mixture Detection, Analysis, and Interpretation

**Leslie D. McCurdy, Ph.D.
Federal Bureau of Investigation
DNA Analysis Unit II**

Bruce Budowle, Constance Fisher, Thomas Hall, Steven Hofstadler,
Alice Isenberg, Thuy-Trang Pennella, Kristin Sannes-Lowery

What Is a Mixture?

Natural

- Heteroplasmy
 - Point / sequence
 - Length

Situational

- Multiple contributors
 - Average number of nucleotide differences between individuals:
 - 8 US Caucasians
 - 14 African Americans
 - 13 Hispanics

Budowle et al. Forensic Science International 1999;103:23-35.

Current Interpretations

- Heteroplasmy
 - Common base at each position?
 - Common length variants detected?
 - Concordant mtDNA types

- Multiple contributors
 - Uninterpretable

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

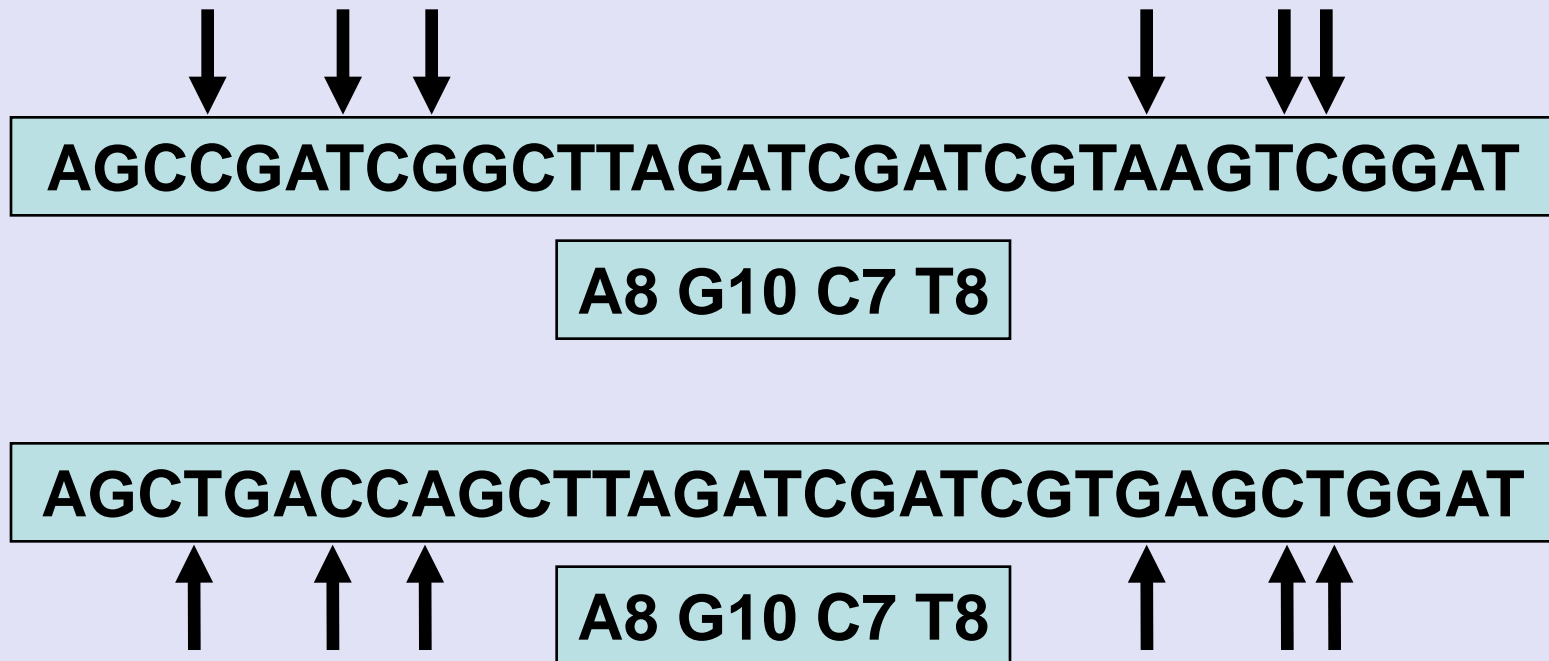
Approaches to mtDNA Mixtures

- Sequencing
- Denaturing High-Performance Liquid Chromatography (DHPLC)
 - Elution & collection of homo- and heteroduplex fractions
- Pyrosequencing
 - Linear relationship between incorporated nucleotides and amount of released light
- Mass Spectrometry

Mass Spectrometry

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

Compensatory Changes

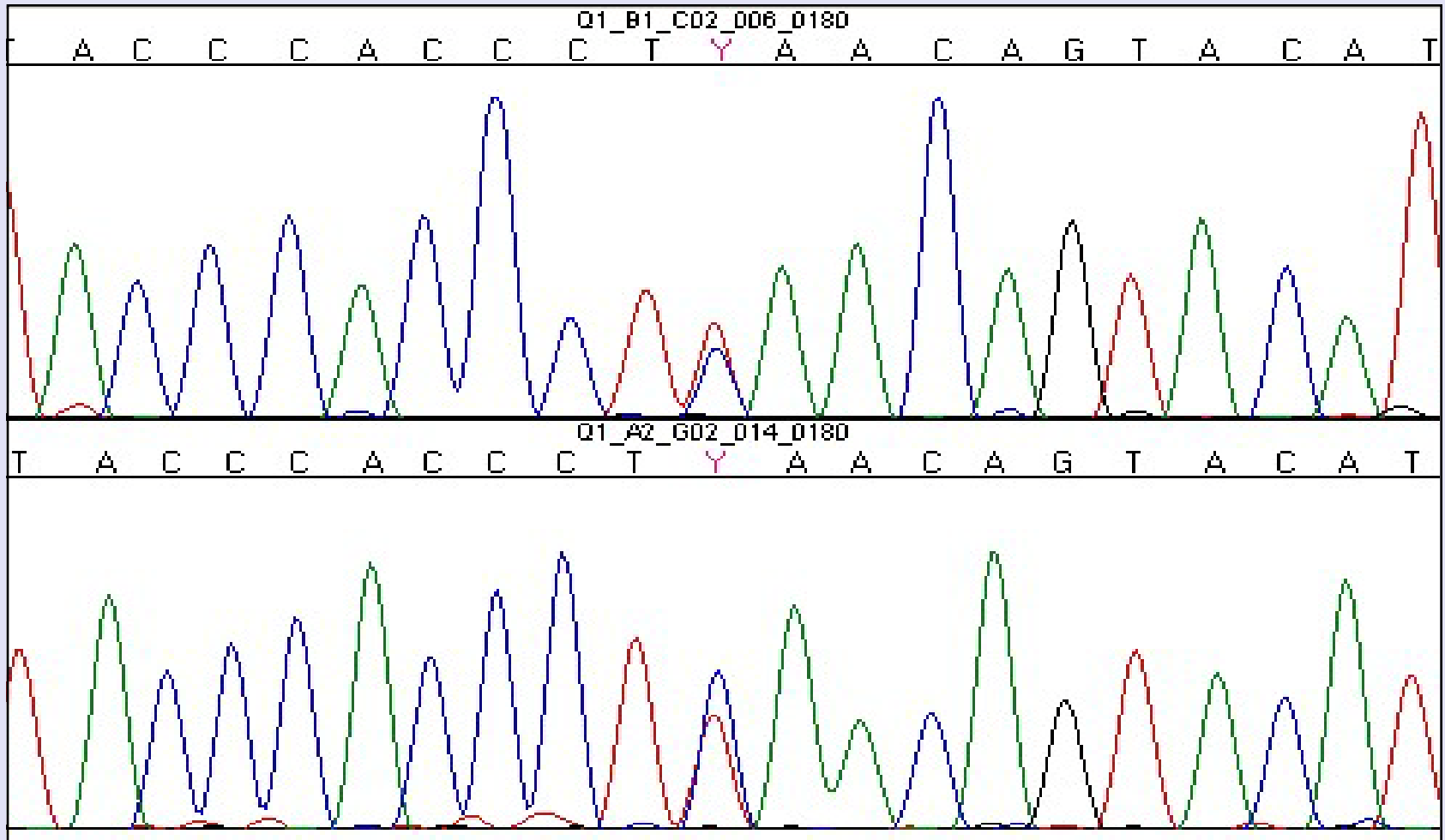


Natural mtDNA Mixtures

Heteroplasmy

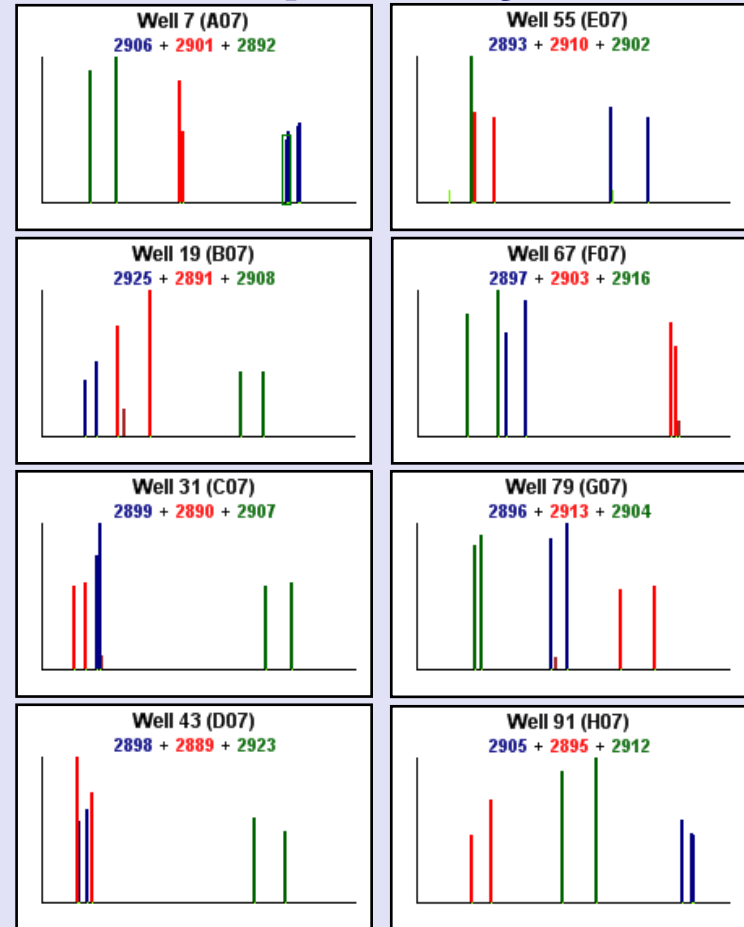
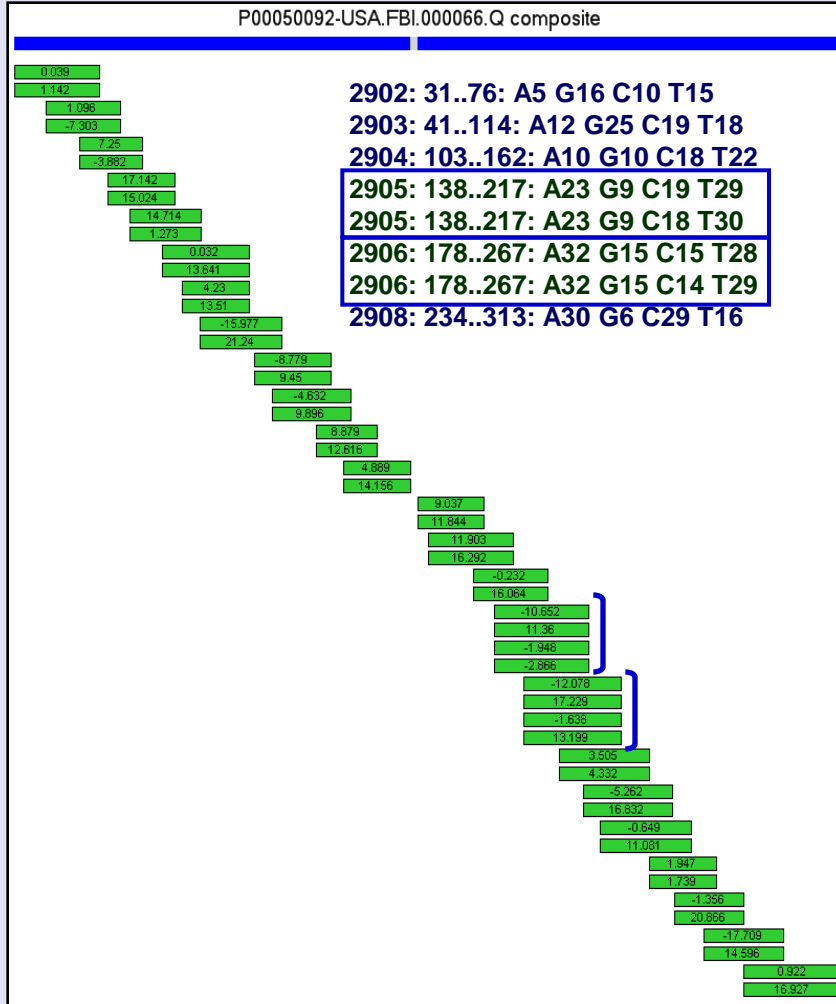
- Use known heteroplasmic mtDNA types
 - Point
 - Length
 - HV1, HV2, HV3
- Perform Ibis mtDNA Assay
- Observe sensitivity and reproducibility
 - Tissue types and within tissue/sample

Point Heteroplasmy

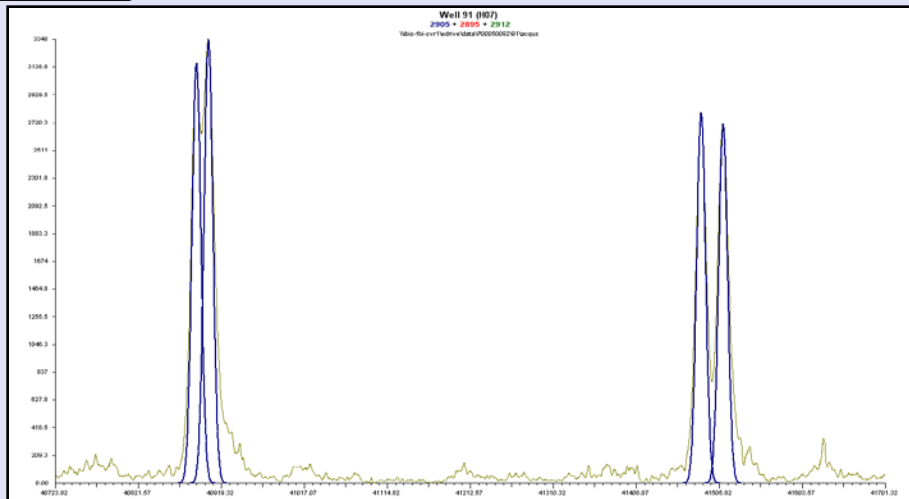


195 Y

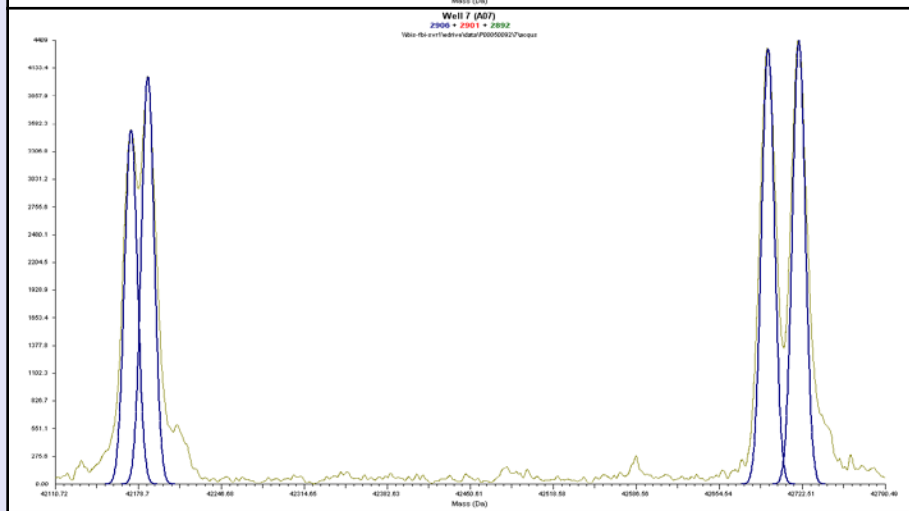
heteroplasmy



NIJ Technology Transition Workshop
 National Institute of Justice

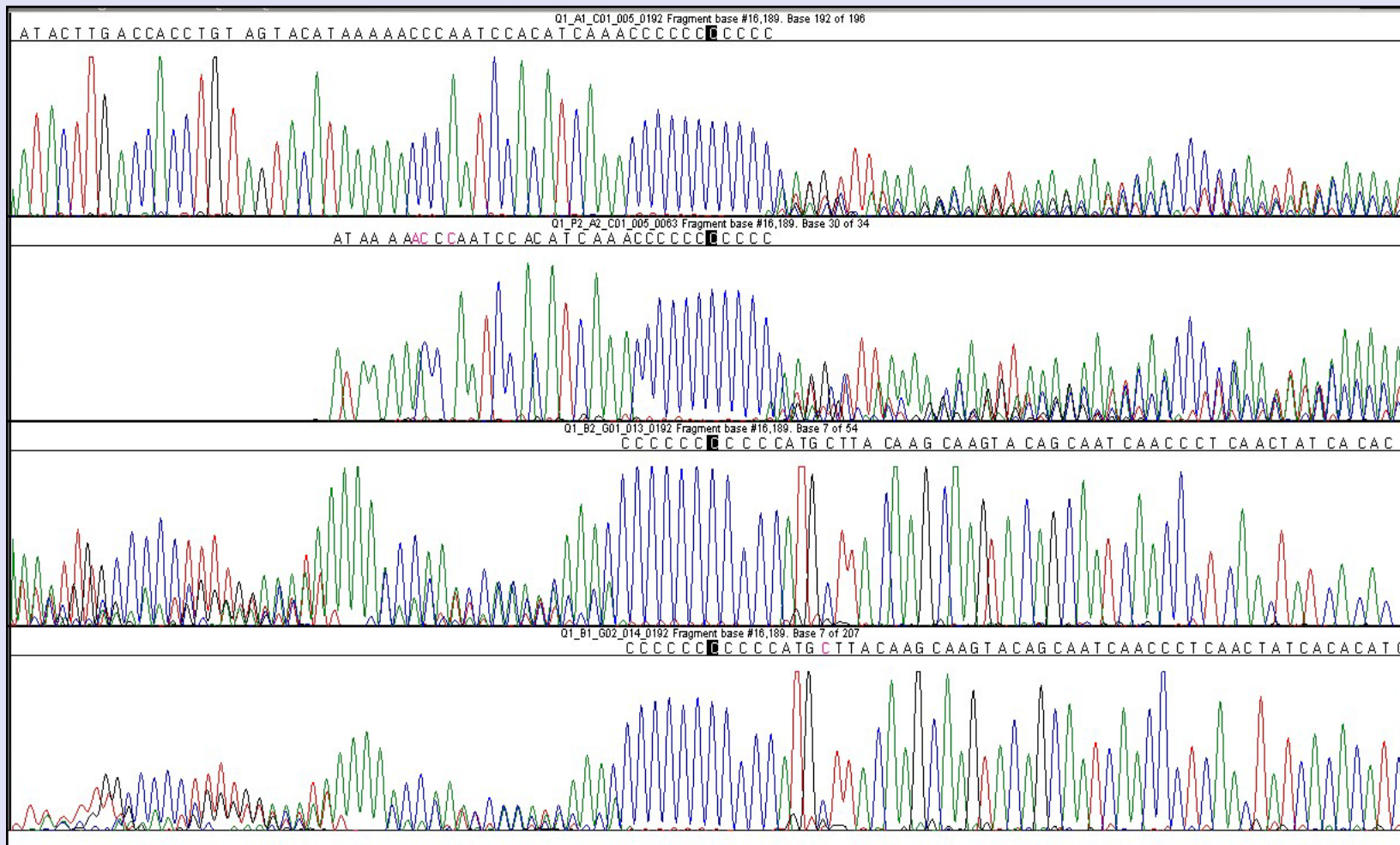


2905: 138..217: A23 G9 **C19 T29**
 2905: 138..217: A23 G9 **C18 T30**
 Well 91 49%
 51%



2906: 178..267: A32 G15 **C15 T28**
 2906: 178..267: A32 G15 **C14 T29**
 Well 7 49%
 51%

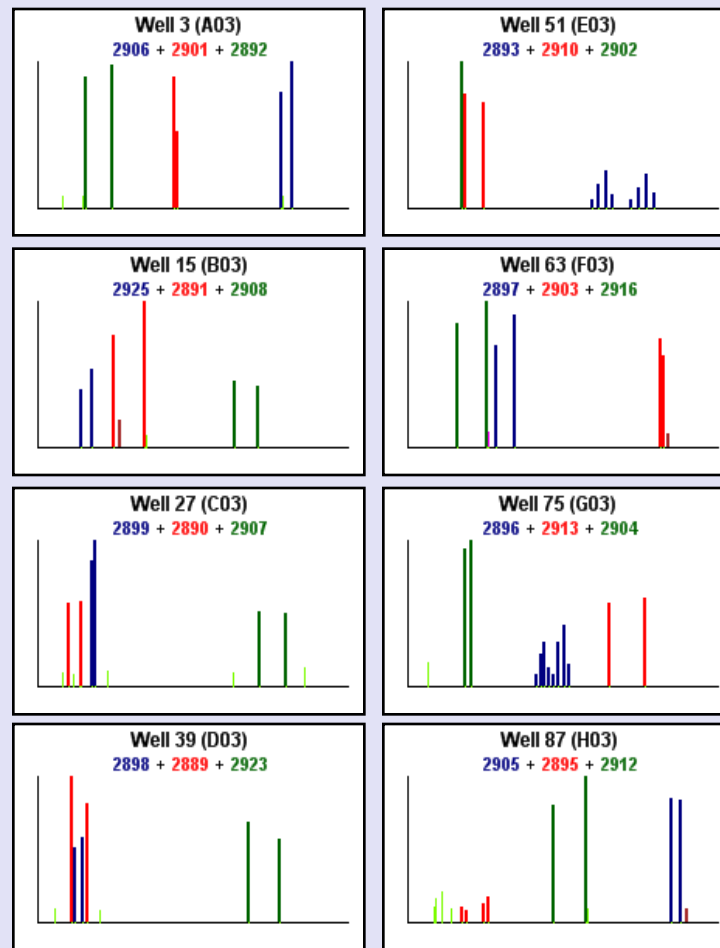
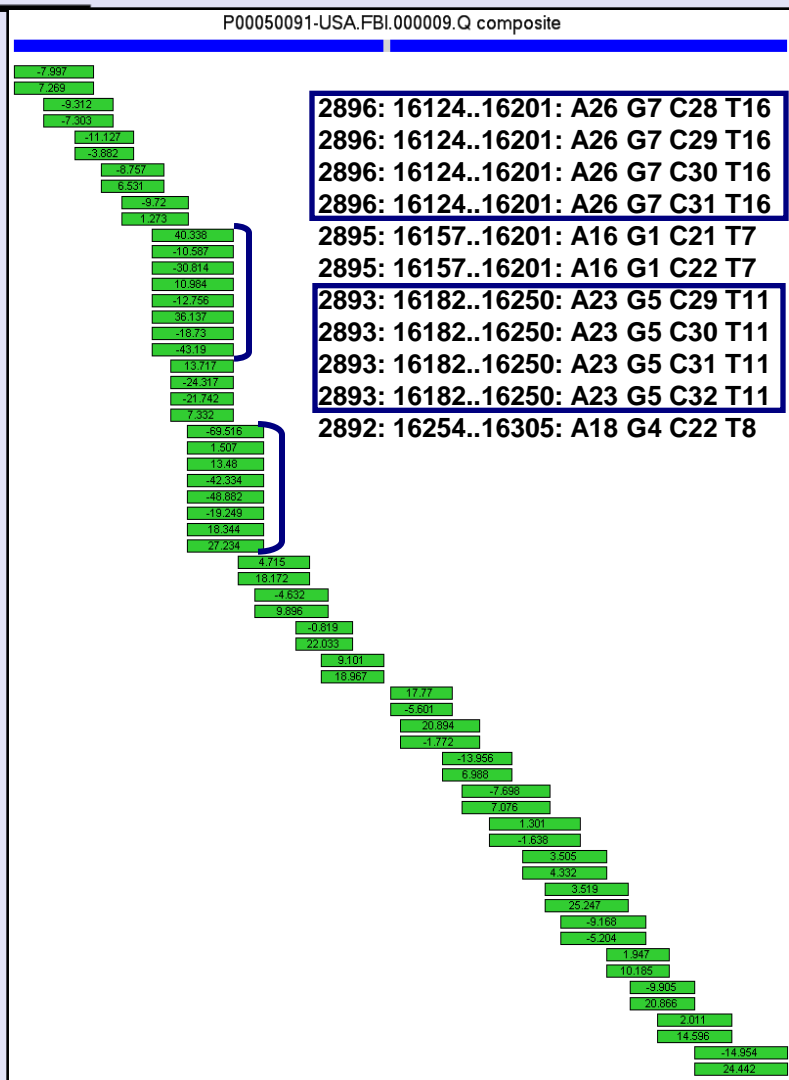
HV1 Length Heteroplasmy



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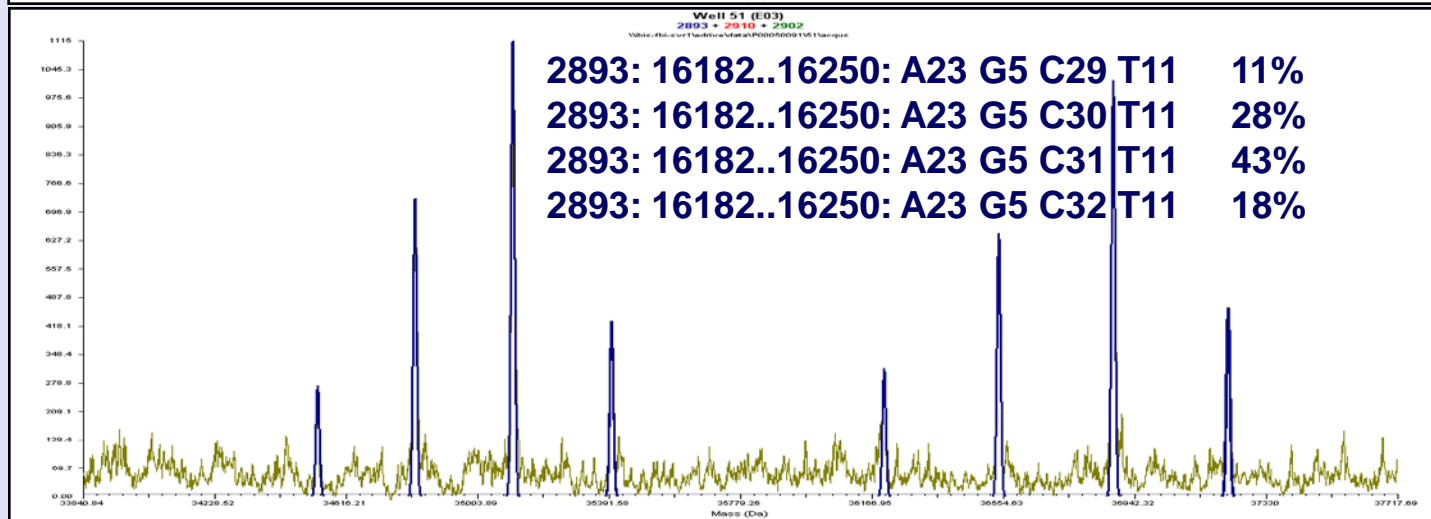
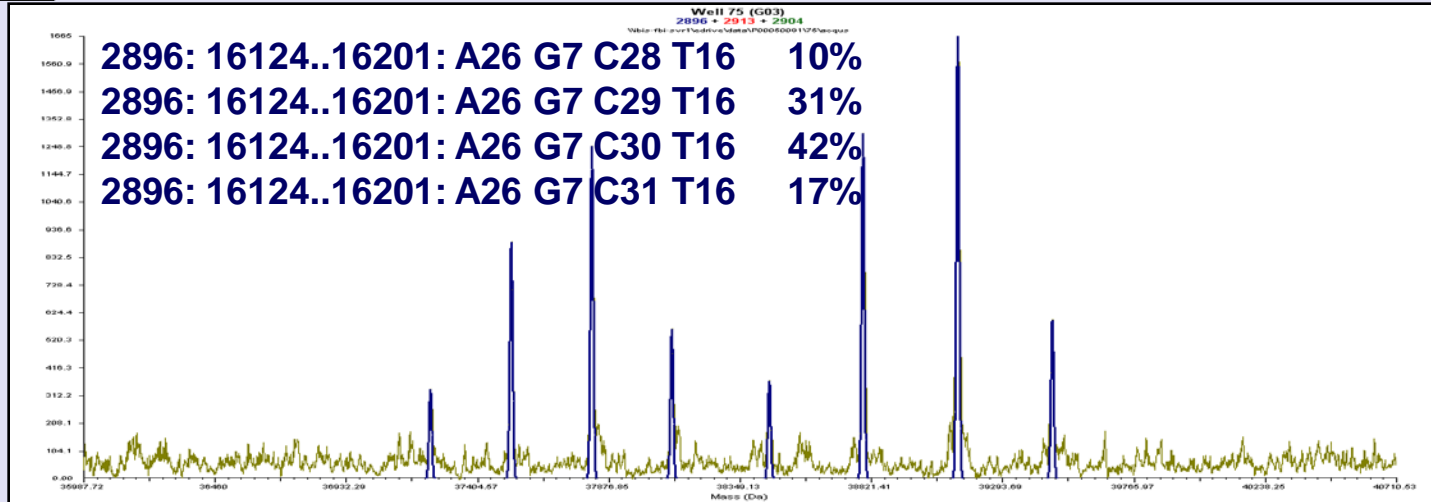
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HV1 length heteroplasmy

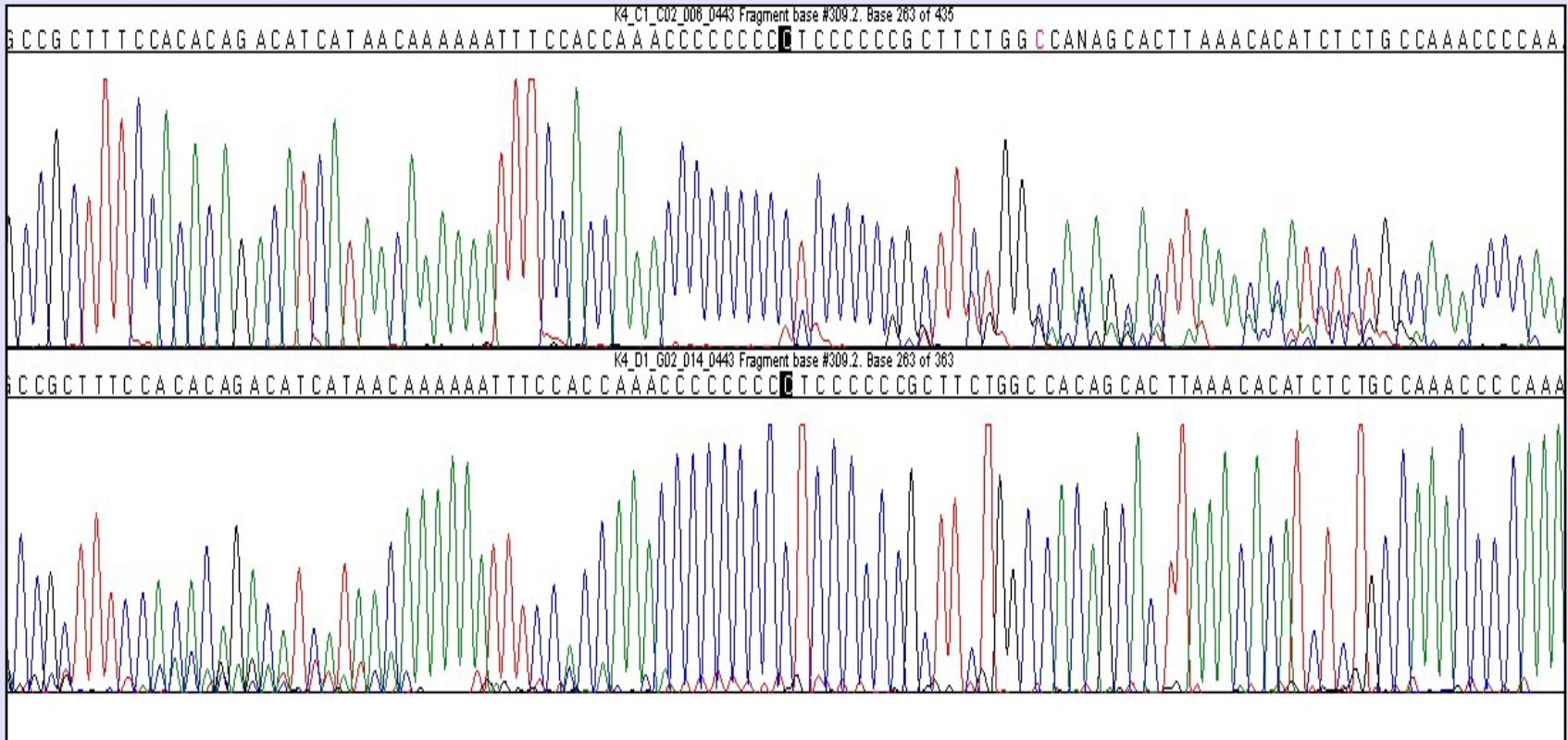


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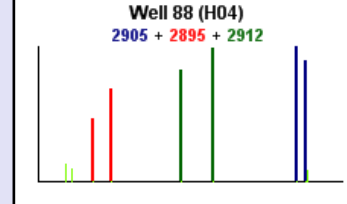
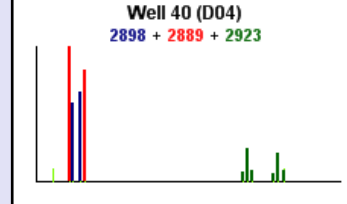
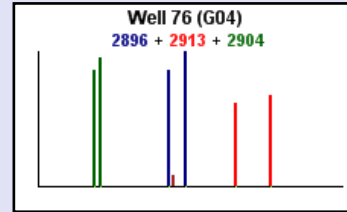
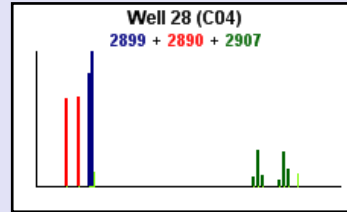
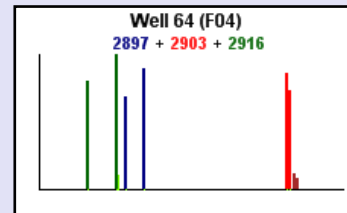
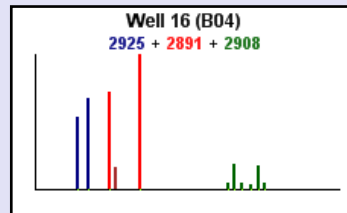
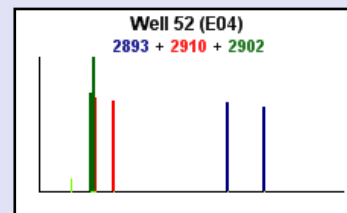
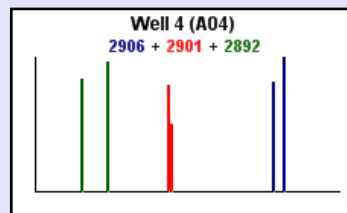
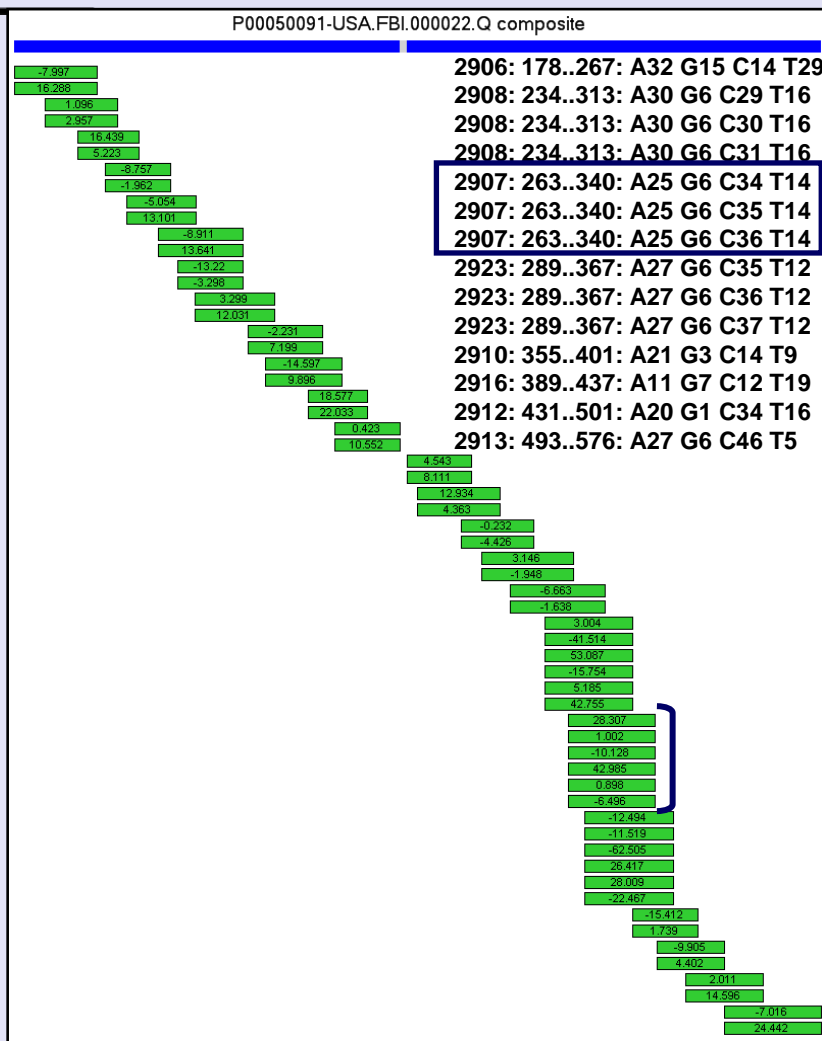
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HV2 Length Heteroplasmy



HV2 length heteroplasmy



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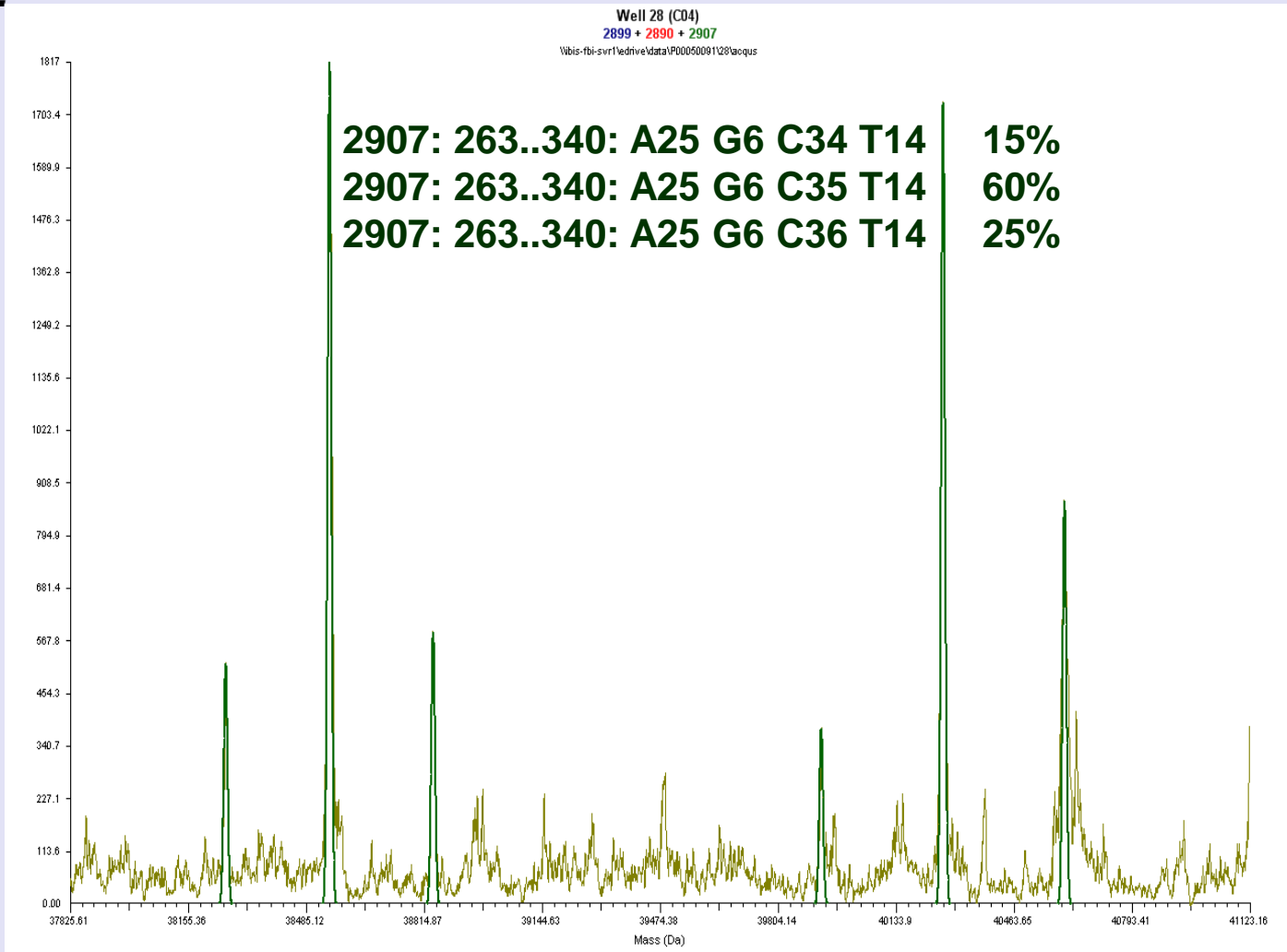
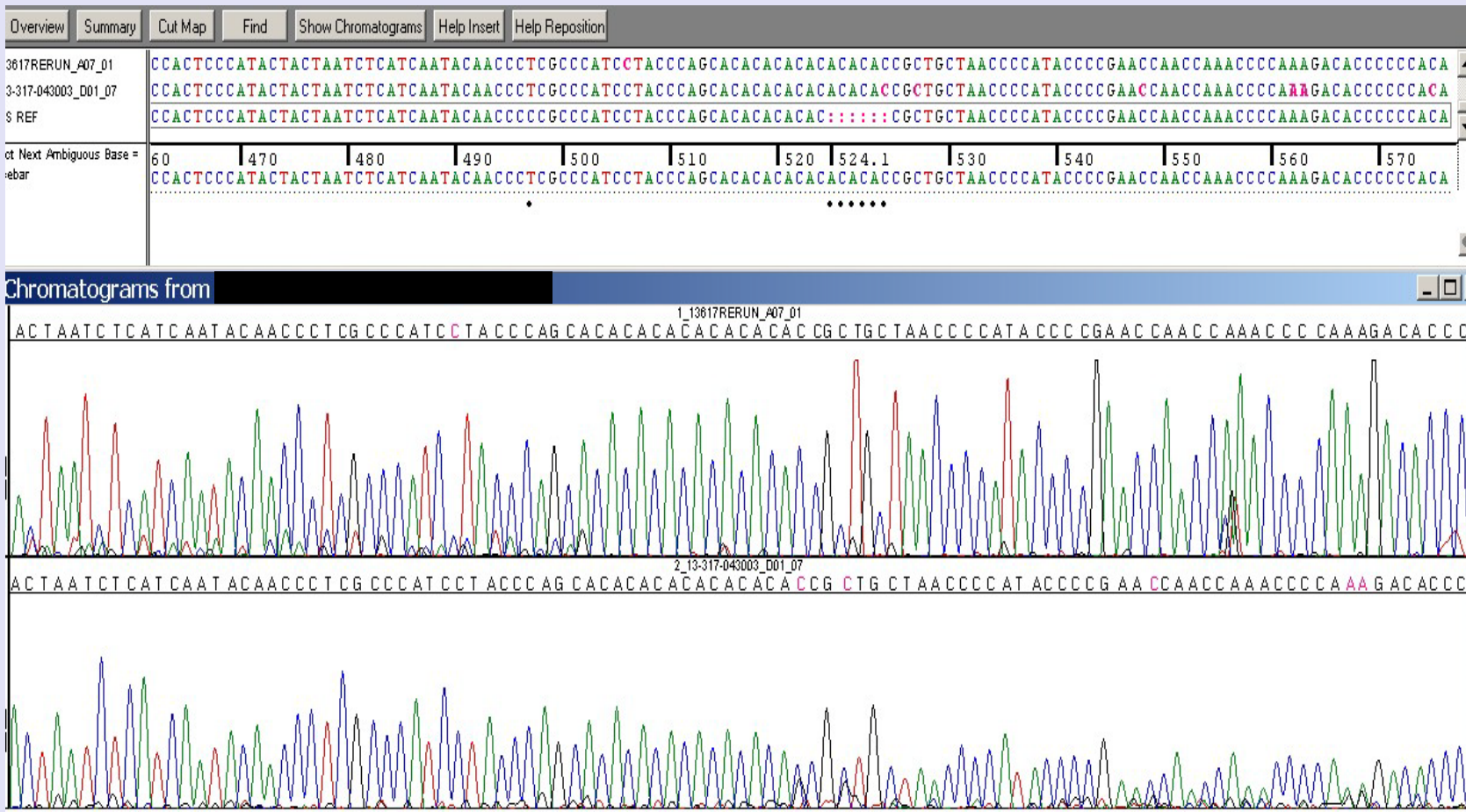
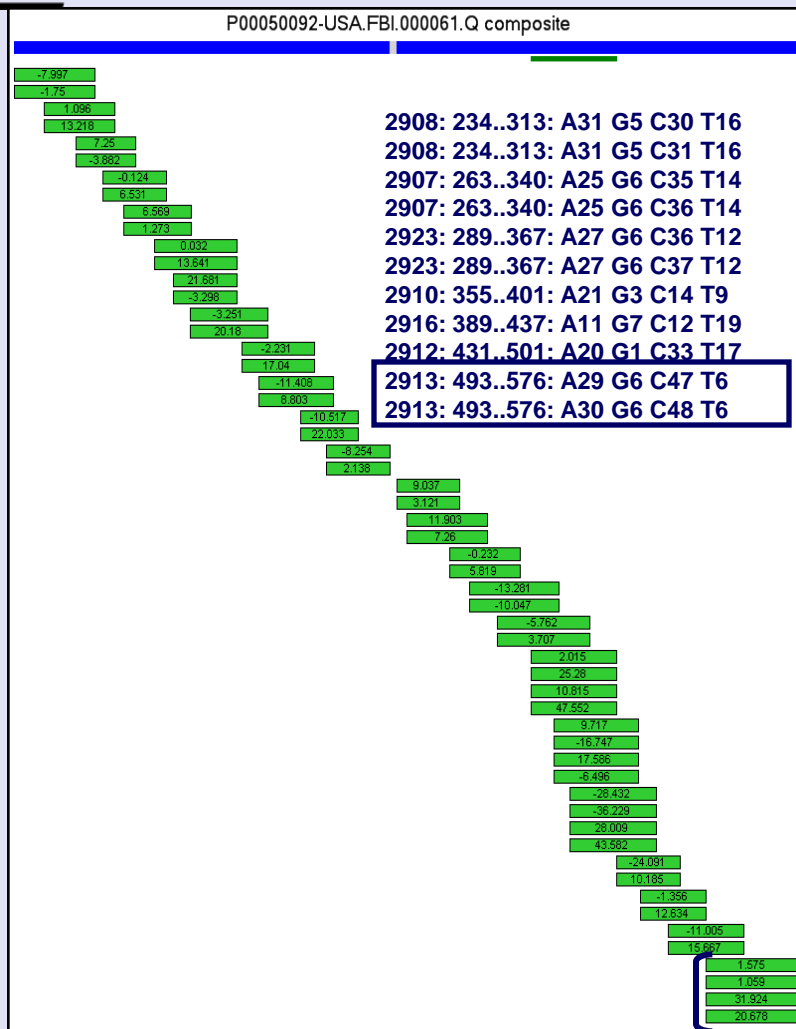


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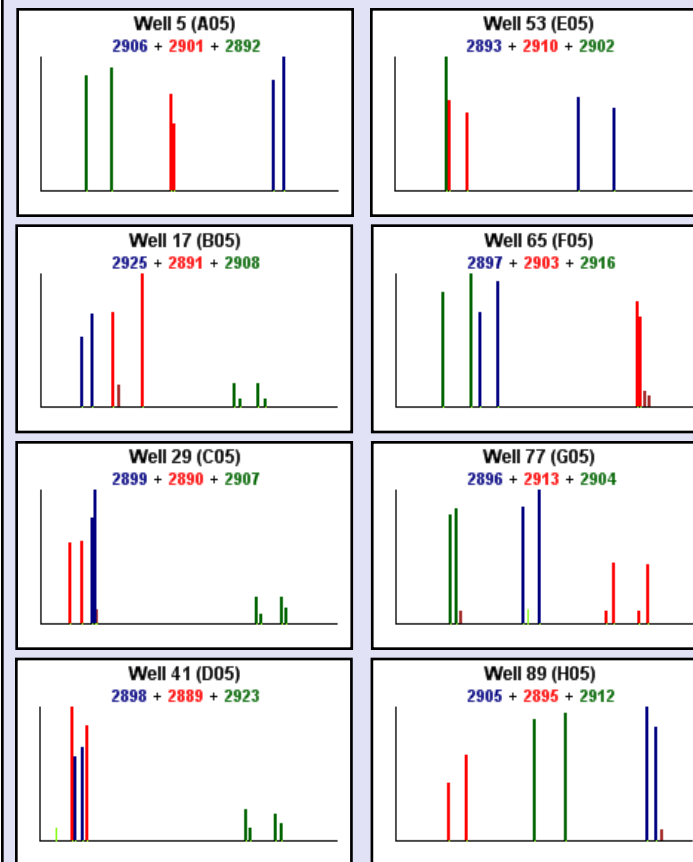
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HV3 CA Repeat





HV3 CA repeat



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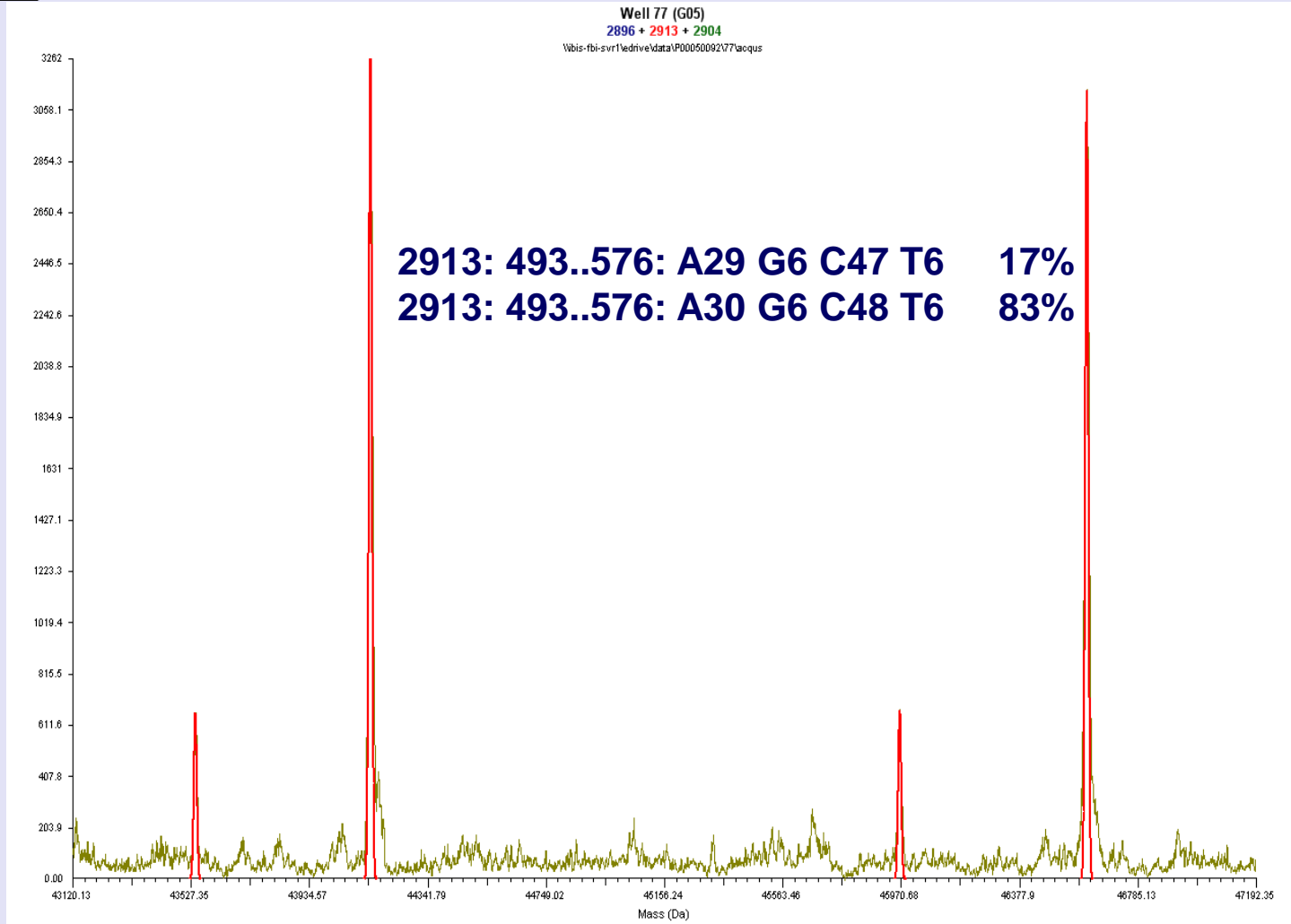
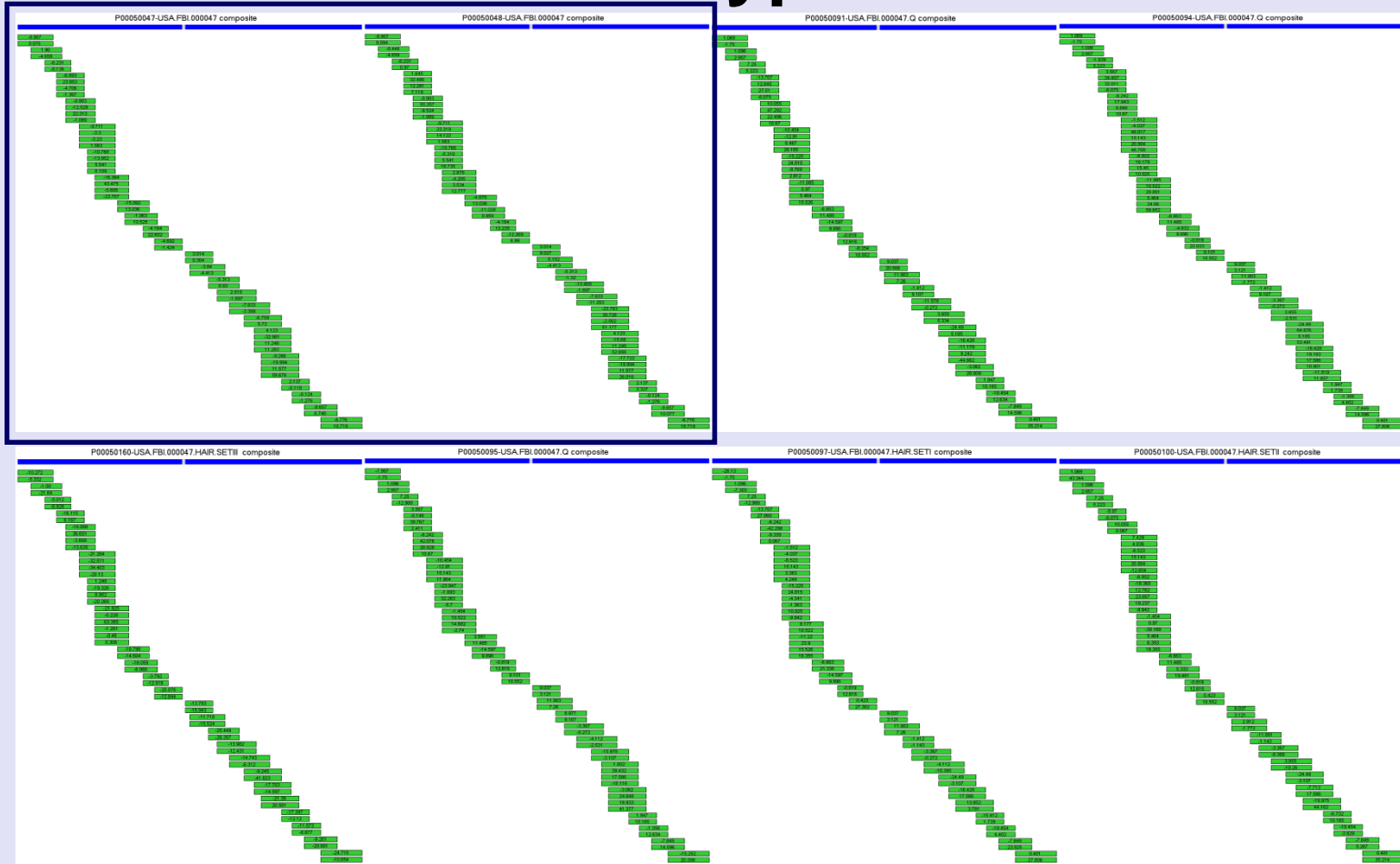


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Concordance Within & Across Tissue Types



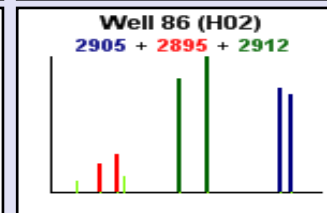
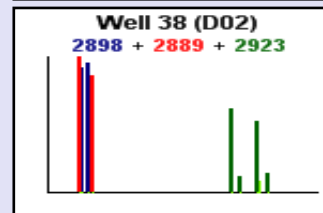
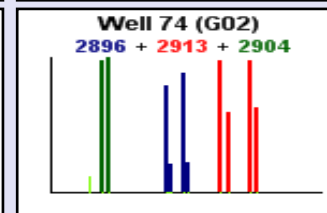
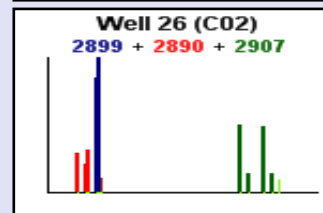
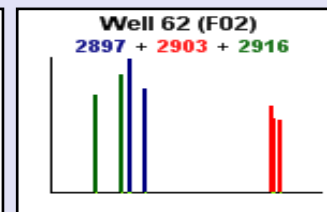
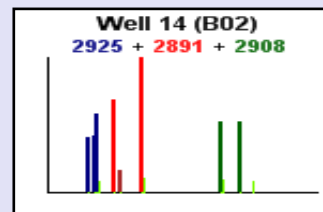
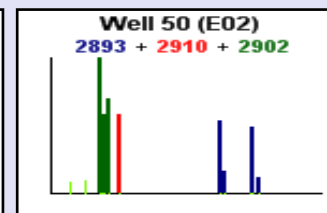
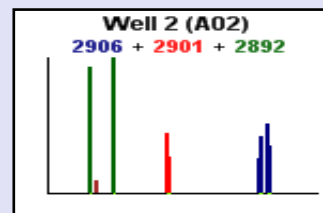
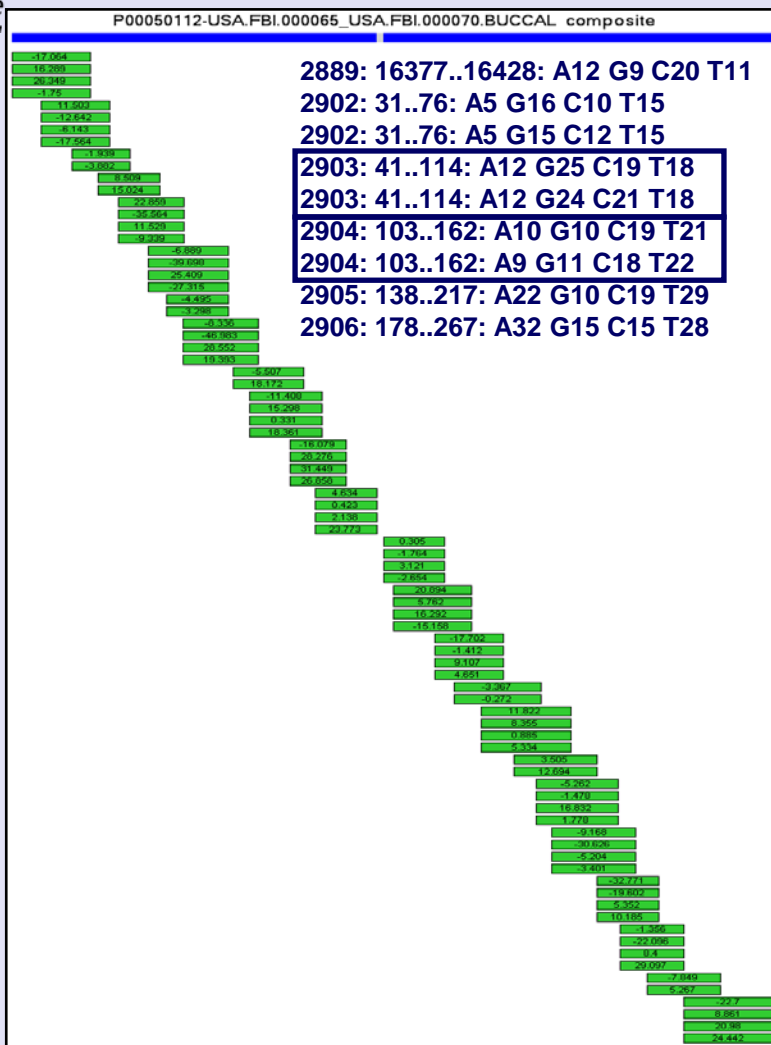
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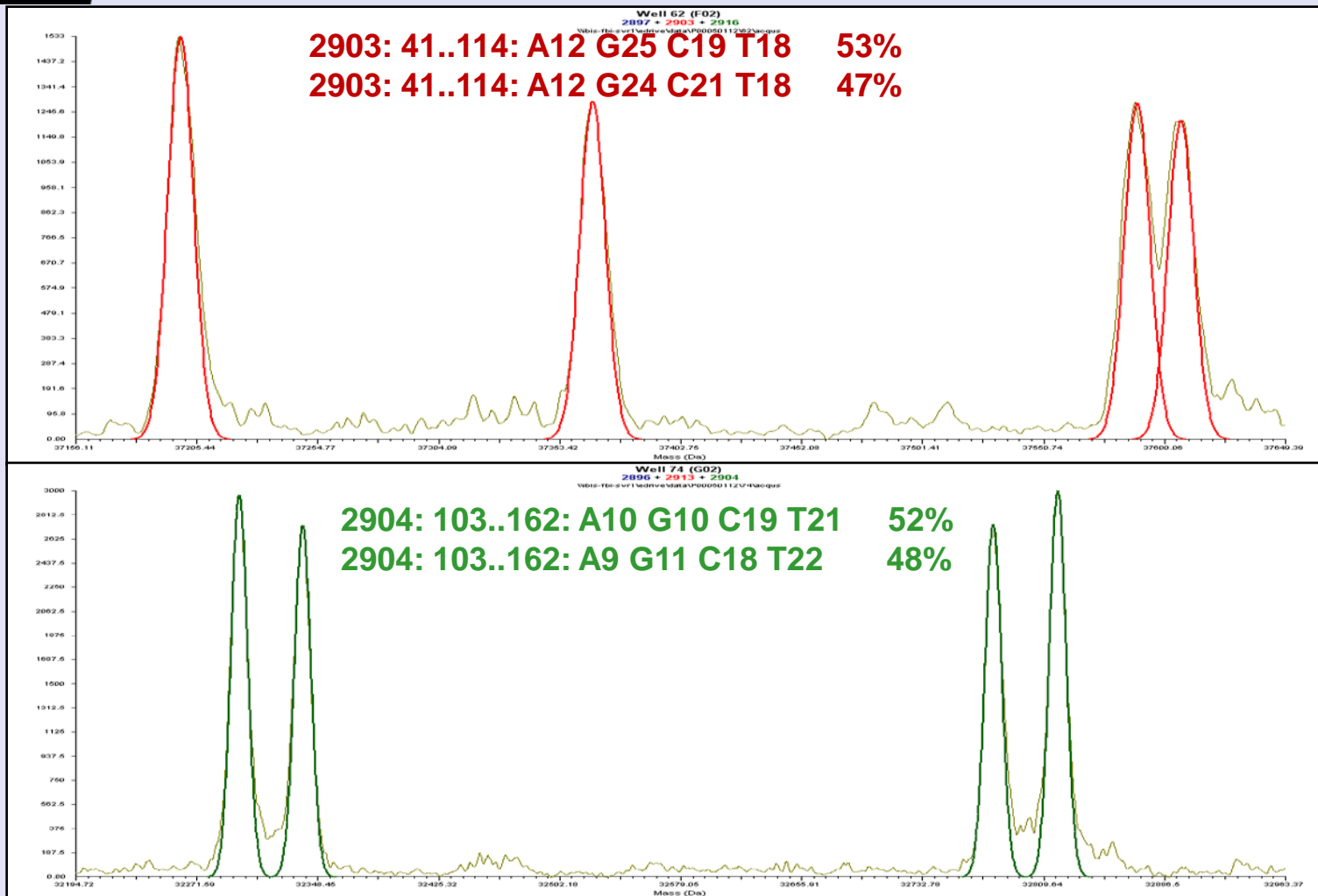
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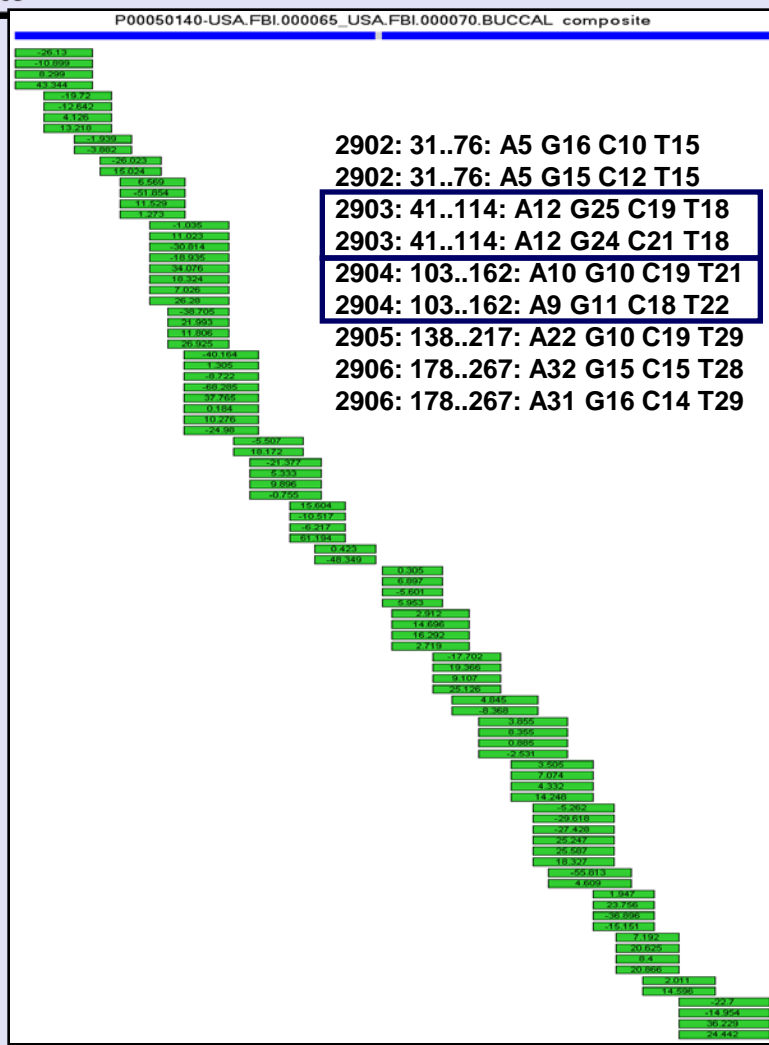
Engineered Mixtures

- Extract known mtDNA types
- Quantify mtDNA copies/ μ L
- Combine mtDNA at predetermined ratios:
 - 50/50
 - 75/25
 - 90/10
- Perform Ibis mtDNA Assay

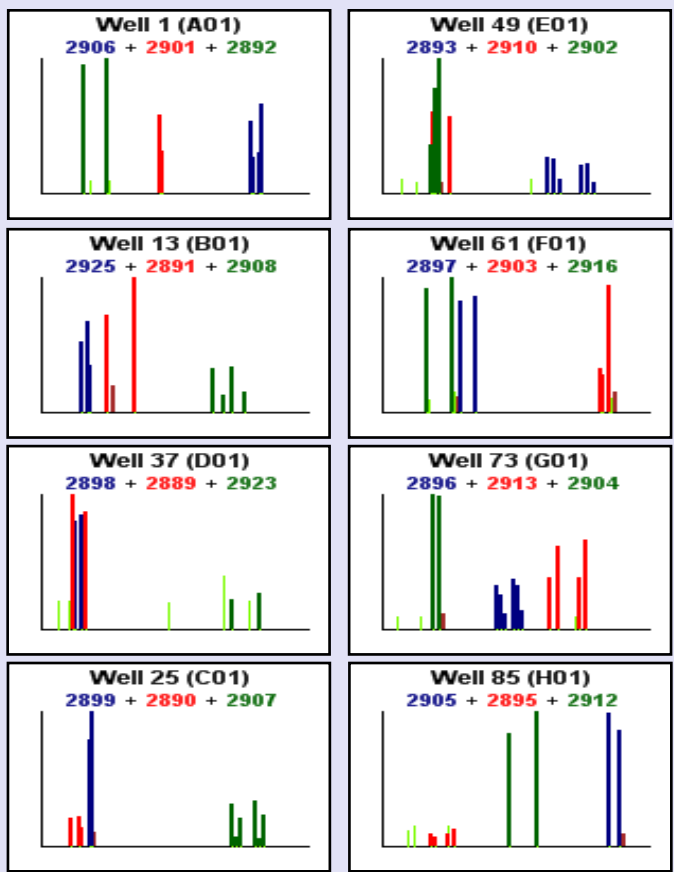
50/50 mixture





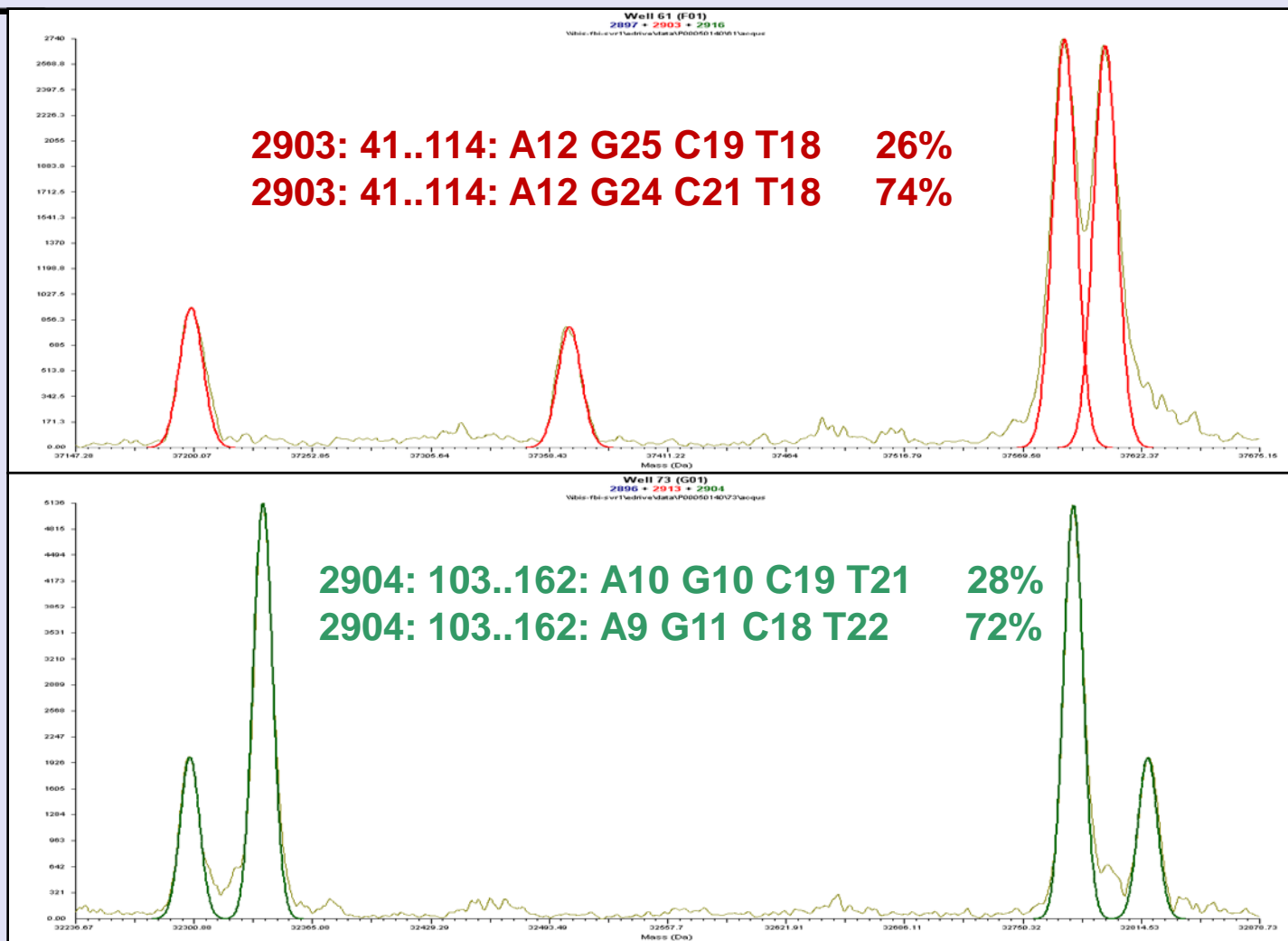


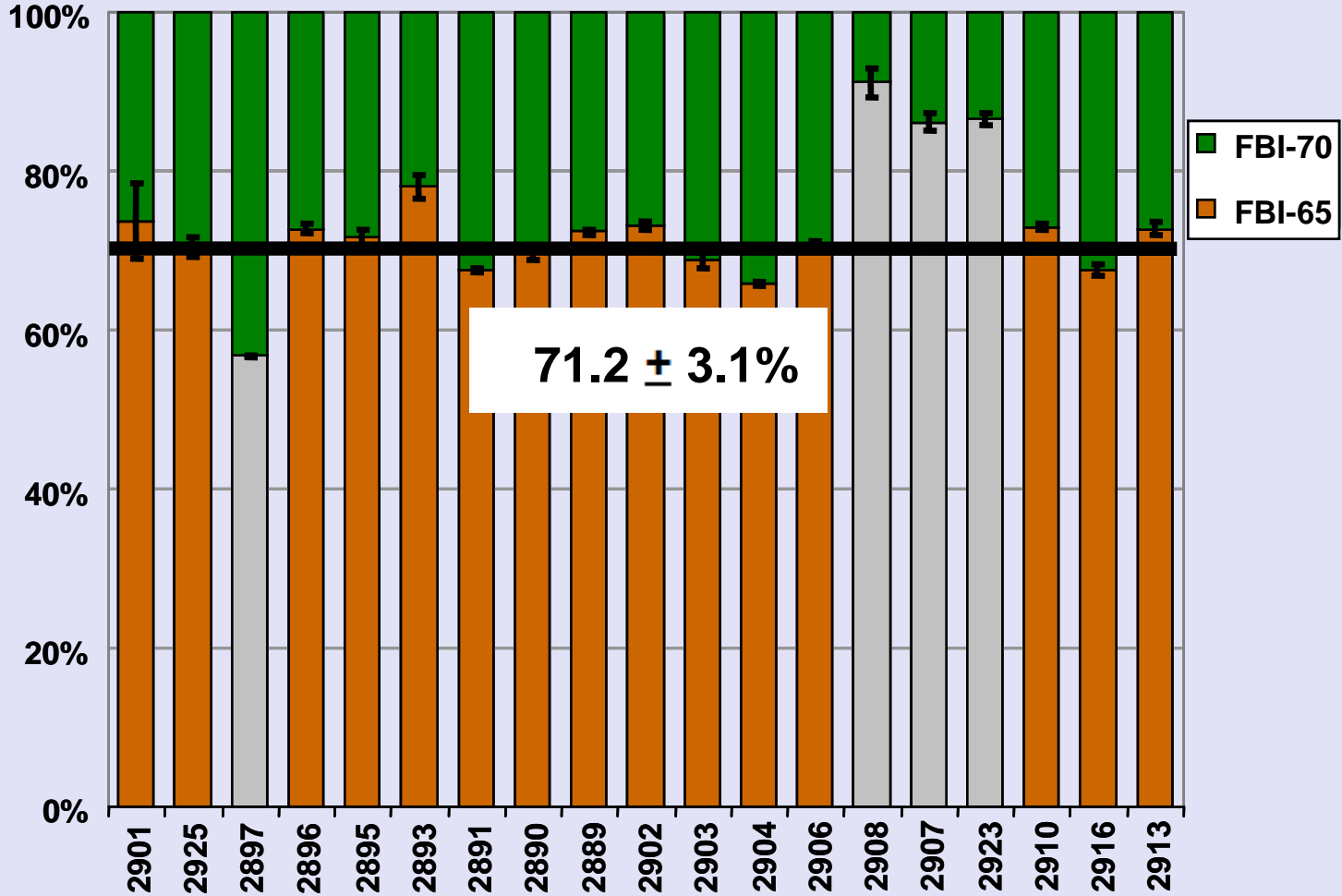
75/25 mixture

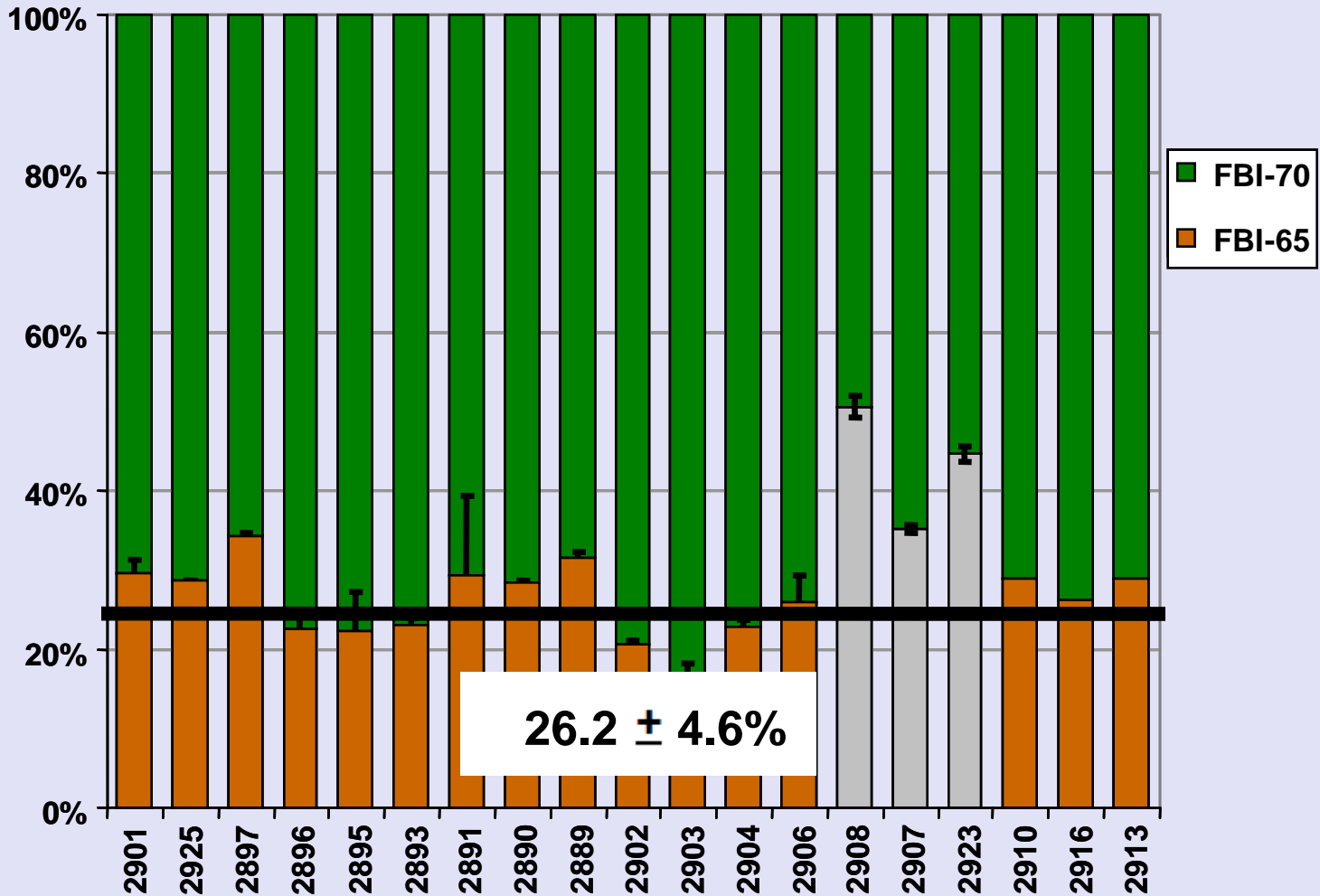


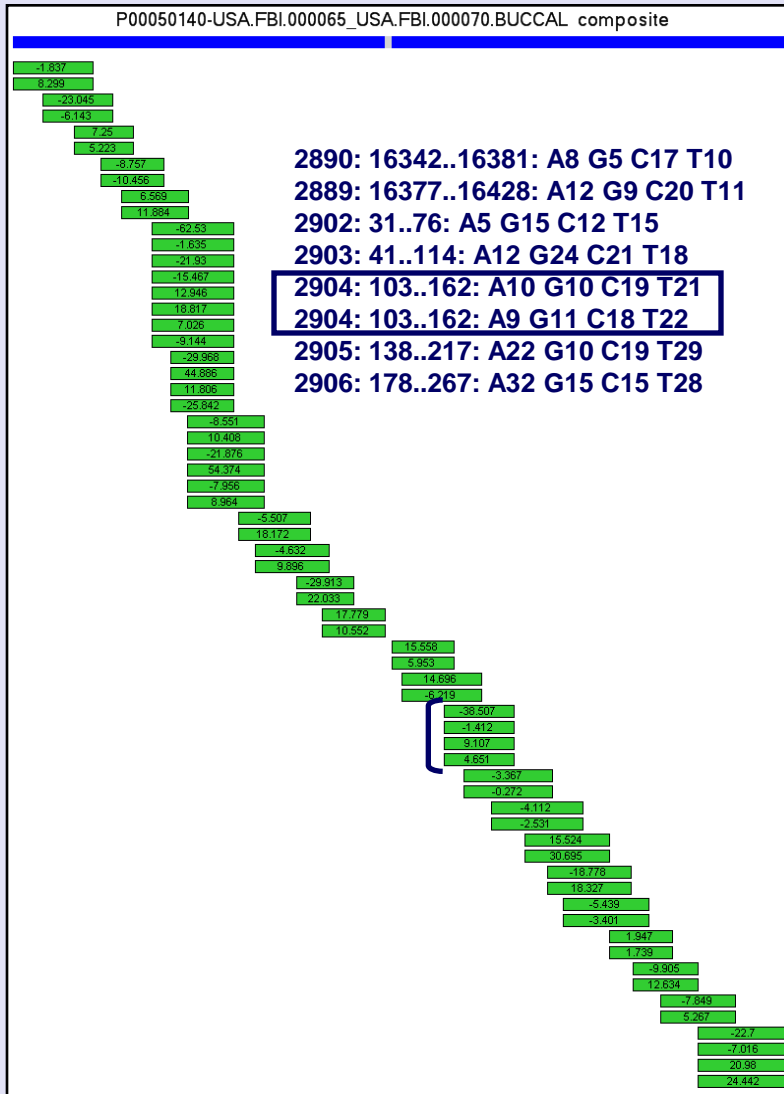
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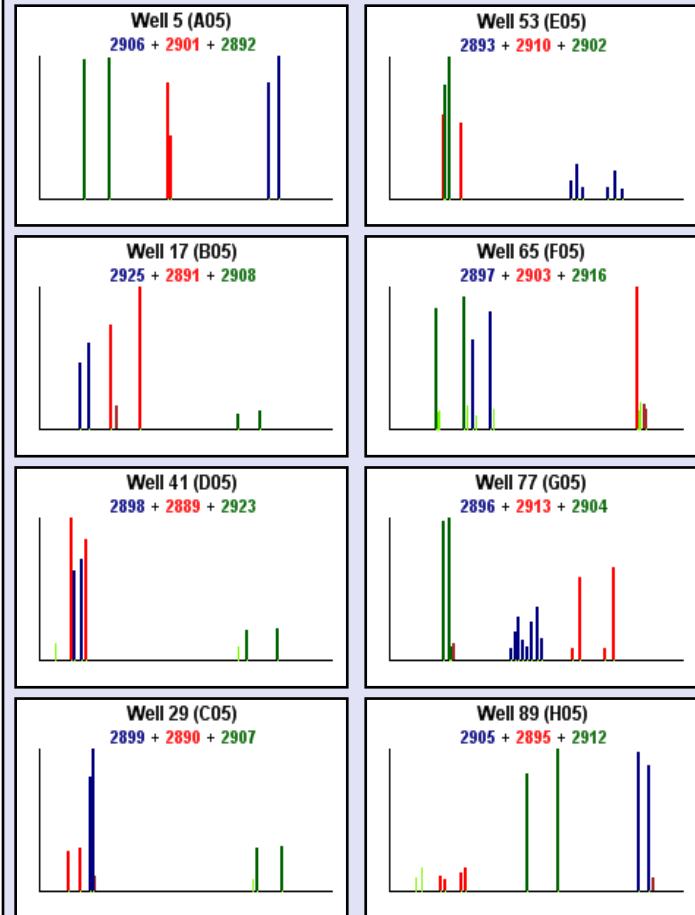








90/10 mixture



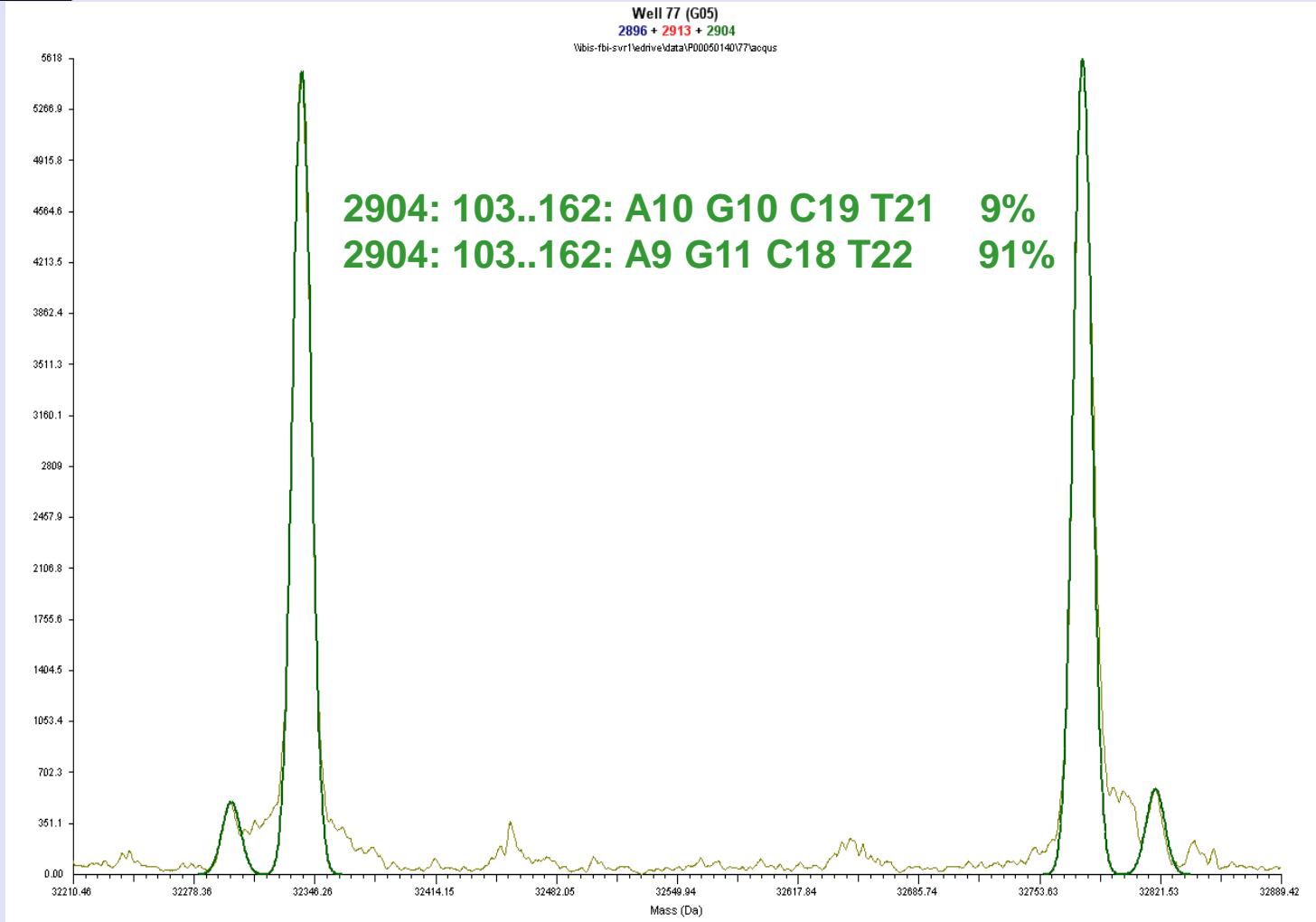
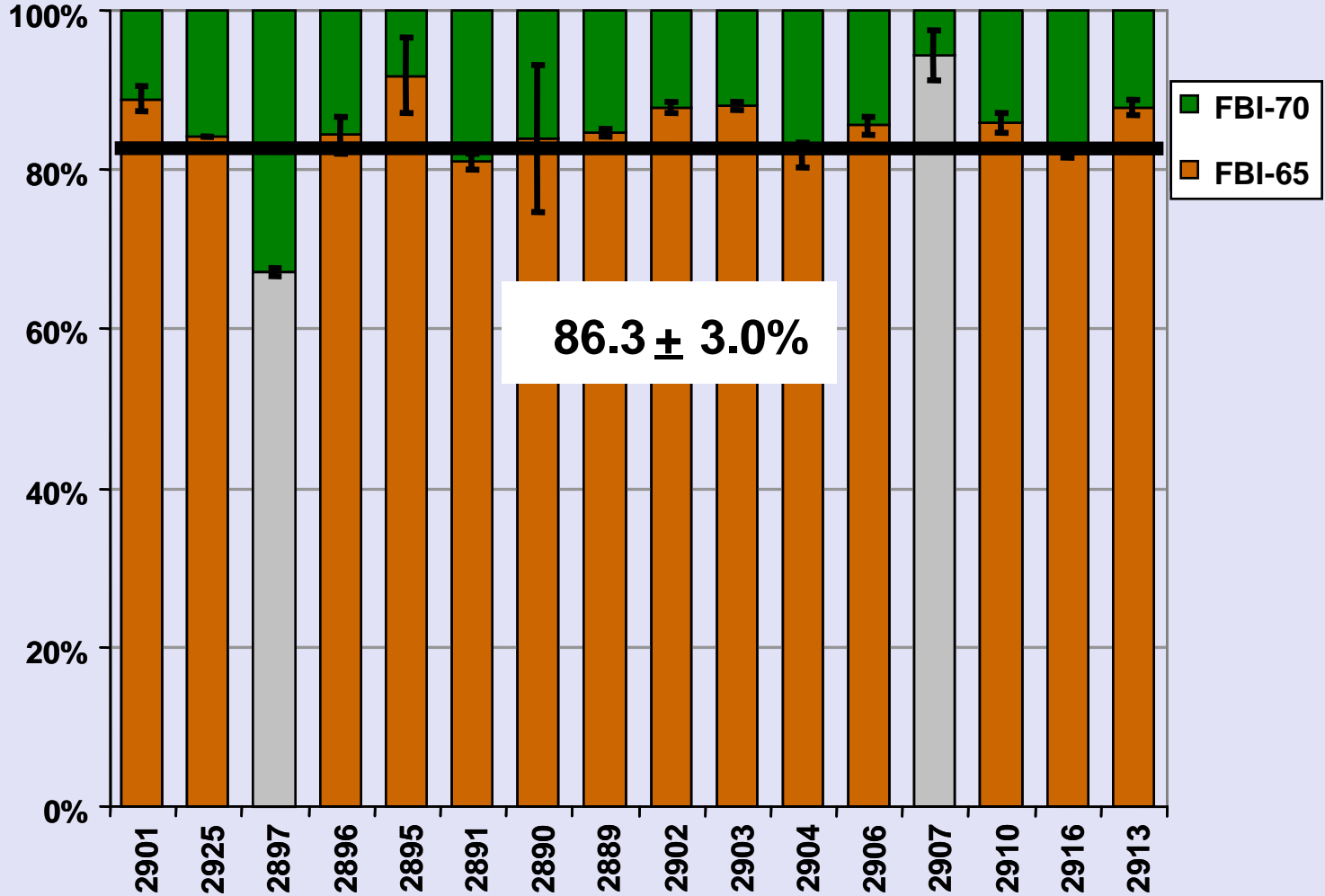
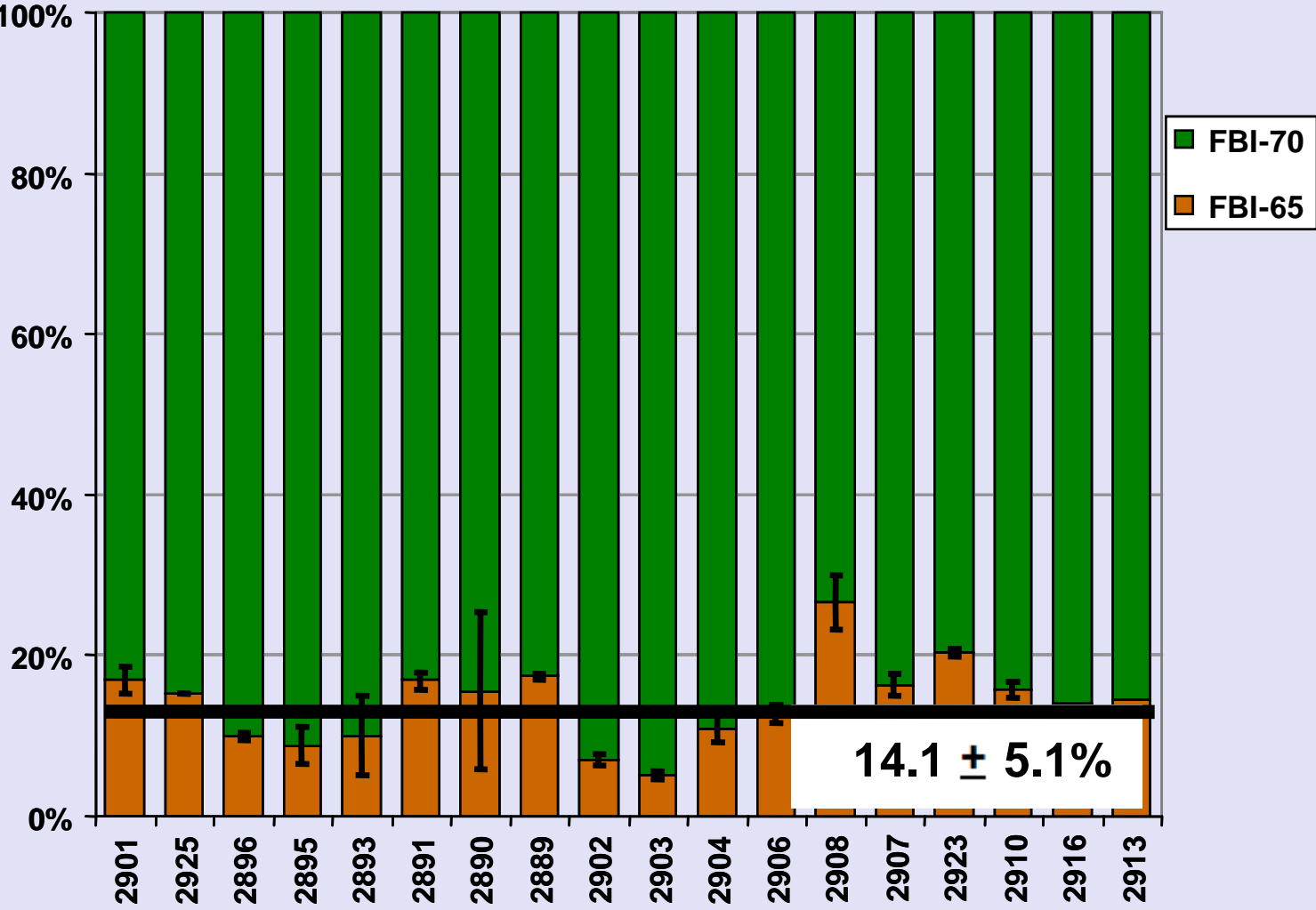


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Interpretation

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, 2908, 2907, 2923, 2913

Engineered

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



Advantages

- Detection of low-level species
 - Sequencing: 15-20%
 - Mass spec: approx 10%
- Resolution of mixed mtDNA components
 - Single technical/analytical procedure
- Multiplex assay
 - Adjacent primer pairs may provide “confirmation” of observations
 - No need for additional procedures

Considerations

- Length heteroplasmy regions
 - Signal splitting may result in artificial inflation of components
- Point heteroplasmy vs. multiple contributors
 - Avg. # of differences between people
- Amplicons not displaying differences
 - Assume concordant types or treat as wildcard?
- Current experiments used 2 component mixture
 - Need to assess more than 2 contributors



Summary

- Sensitive and reproducible
 - Heteroplasmy
 - Engineered
 - 10% minor component
- Quantitation varied by:
 - 5% for 75/25 mixtures
 - 3.5% for 90/10 mixtures
- Subtraction and quantitation of multiple contributors



Collaborators

FBI

- Bruce Budowle
- Connie Fisher
- Alice Isenberg
- Thuy Pennella

Ibis BioSciences

- Steve Hofstadler
- Tom Hall
- Kristin Lowery



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Contact information:

Leslie D. McCurdy, Ph.D.
Federal Bureau of Investigation
DNA Analysis Unit II

leslie.mccurdy@ic.fbi.gov

703-632-7801