Mitochondrial DNA Mixture Detection, Analysis, and Interpretation

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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

Current Interpretations

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

• Multiple contributors
  – Uninterpretable
Challenges

• Heteroplasm 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

AGCCGATCGGCTTTAGATCGATCGTAAGTGGAT

A8 G10 C7 T8

AGCTGACCAGCTTTAGATCGATCGTGAGCTGGAT

A8 G10 C7 T8

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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

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195 Y heteroplasmy

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Well 91
49%
51%

Well 7
49%
51%

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HV1 Length Heteroplasmy

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HV1 length heteroplasy

2896: 16124..16201: A26 G7 C28 T16
2896: 16124..16201: A26 G7 C29 T16
2896: 16124..16201: A26 G7 C30 T16
2896: 16124..16201: A26 G7 C31 T16
2895: 16157..16201: A16 G1 C21 T7
2895: 16157..16201: A16 G1 C22 T7
2893: 16182..16250: A23 G5 C29 T11
2893: 16182..16250: A23 G5 C30 T11
2893: 16182..16250: A23 G5 C31 T11
2893: 16182..16250: A23 G5 C32 T11
2892: 16254..16305: A18 G4 C22 T8

Well 3 (E03) 2906 + 2910 + 2902
Well 51 (E03) 2903 + 2910 + 2902
Well 15 (E03) 2905 + 2904 + 2908
Well 63 (E03) 2907 + 2903 + 2916
Well 27 (E03) 2909 + 2906 + 2907
Well 75 (E03) 2906 + 2913 + 2904
Well 39 (E03) 2908 + 2909 + 2923
Well 87 (E03) 2905 + 2905 + 2912

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2896: 16124..16201: A26 G7 C28 T16  10%
2896: 16124..16201: A26 G7 C29 T16  31%
2896: 16124..16201: A26 G7 C30 T16  42%
2896: 16124..16201: A26 G7 C31 T16  17%

2893: 16182..16250: A23 G5 C29 T11  11%
2893: 16182..16250: A23 G5 C30 T11  28%
2893: 16182..16250: A23 G5 C31 T11  43%
2893: 16182..16250: A23 G5 C32 T11  18%
HV2 Length Heteroplasmy

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HV2 length heteroplasmy

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2907: 263..340: A25 G6 C34 T14 15%
2907: 263..340: A25 G6 C35 T14 60%
2907: 263..340: A25 G6 C36 T14 25%
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HV3 CA repeat

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Concordance Within & Across Tissue Types

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Engineered Mixtures

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

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2902: 31..76: A5 G16 C10 T15
2902: 31..76: A5 G15 C12 T15
2903: 41..114: A12 G25 C19 T18
2903: 41..114: A12 G24 C21 T18
2904: 103..162: A10 G10 C19 T21
2904: 103..162: A9 G11 C18 T22
2905: 138..217: A22 G10 C19 T29
2906: 178..267: A32 G15 C15 T28
2906: 178..267: A31 G16 C14 T29

75/25 mixture

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26.2 ± 4.6%

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2890: 16342..16381: A8 G5 C17 T10
2889: 16377..16428: A12 G9 C20 T11
2902: 31..76: A5 G15 C12 T15
2903: 41..114: A12 G24 C21 T18
2904: 103..162: A10 G10 C19 T21
2904: 103..162: A9 G11 C18 T22
2905: 138..217: A22 G10 C19 T29
2906: 178..267: A32 G15 C15 T28

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2904: 103..162: A10 G10 C19 T21 9%
2904: 103..162: A9 G11 C18 T22 91%
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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

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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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