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AMERICAN ACADEMY *of* **FORENSIC SCIENCES**

Alternative Methods for Human Identification: Mitochondrial DNA Base Composition Profiling by ESI-TOF Mass Spectrometry

Kevin Kiesler

Eric Pokorak, Thomas Callaghan PhD, Peter Vallone PhD

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Disclaimer

This presentation will discuss the PLEX-ID ESI-TOF mass spectrometer developed by Ibis Biosciences and marketed by Abbott Molecular

The PLEX-ID has been voluntarily recalled from the market due to reliability issues reported by clinical customers
- **It is being redesigned for re-release in the future**

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Points of view are those of the presenters and do not necessarily represent the official position of the Department of Commerce, the National Institute of Standards and Technology, or the U.S. Department of Justice.

Presentation Outline

- Introduction & background
- Why mass spectrometry?
- PLEX-ID mtDNA 2.0 Assay
- Concordance study
- Haplotype diversity

Assessment Experiments

- Sensitivity
  - Dilution series of three templates
  - (4, 8, 20, 40) pg total DNA input
  - Average % of amplicons detected
    - 72.4% at 4 pg DNA input
    - 85.1% at 8 pg DNA input
    - 96.0% at 20 pg DNA input
    - 98.8% at 40 pg DNA input
  - Manufacturer recommends 200 pg DNA input

- Contamination
  - Plate layout designed to evaluate reagents, fluidics, and cleanup carousel
  - Run twice per month for six months
  - No contamination detected

- Concordance
  - Comparing M.S. to sequencing
  - 711 templates analyzed
  - 99.3 % concordance rate (706/711)

- Mixtures
  - Two-component mixtures generated
  - Ratios - 99:1, 19:1, 9:1, 3:1, and 1:1
  - 3:1 mixture was limit of minor component detection

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Full Report Available Online

- http://www.cstl.nist.gov/strbase/NISTpub.htm

NIST Report to the FBI:
Plex-ID Electrospray Time-of-Flight Mass
Spectrometer for Mitochondrial DNA
Base Composition Profiling

Experiments performed and report written by: Kevin Kiesler, M.S. (NIST)

Under the direction of: Dr. Peter Vallone (NIST)

Editorial contributors:

Dr. Peter Vallone (NIST)

Dr. John Butler (NIST)

Dr. Thomas Callaghan (FBI)

Eric Pokorak (FBI)

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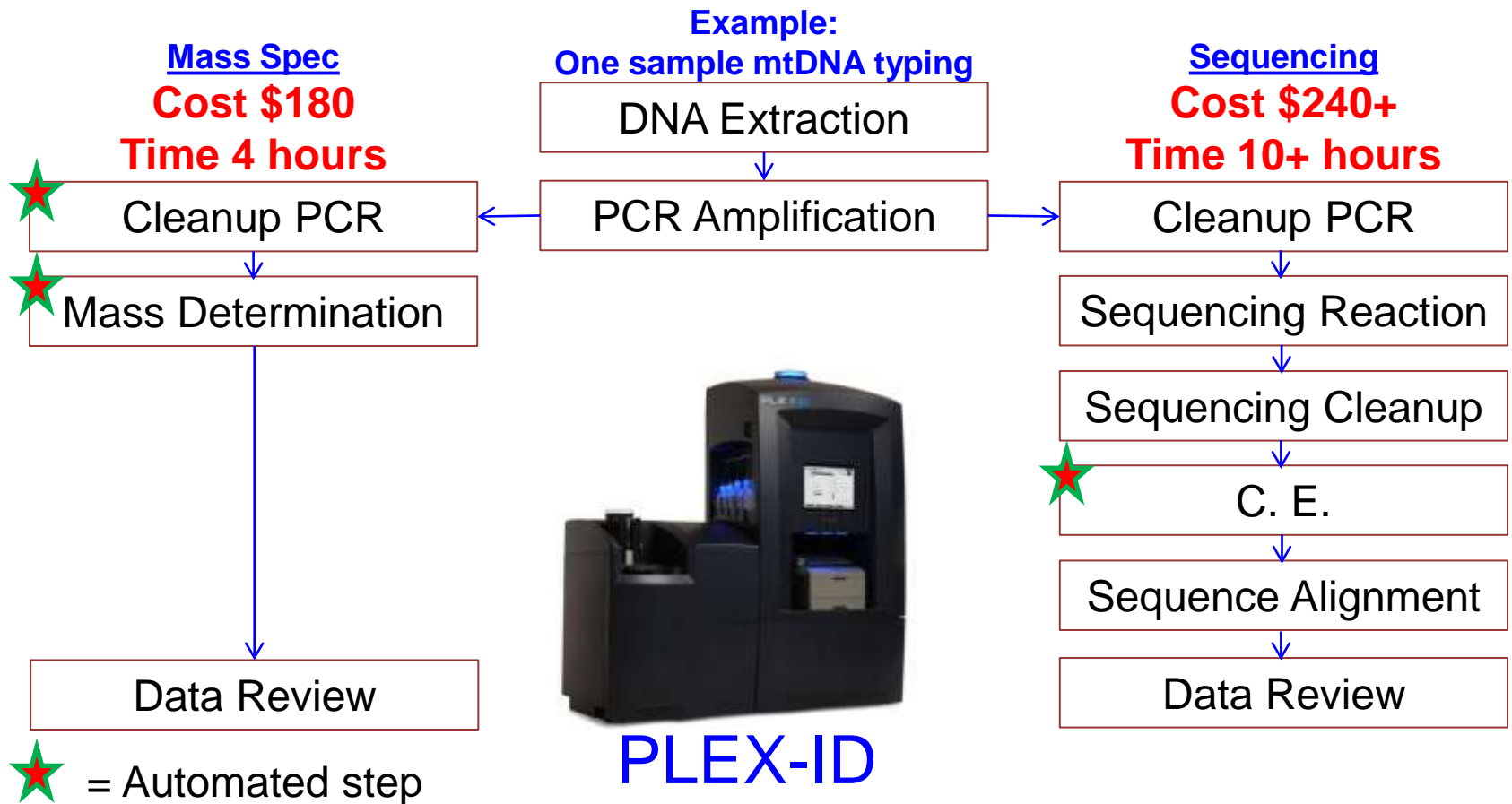
Mitochondrial DNA for Human ID

- Mitochondrial DNA (mtDNA) **sequence** information has been used in forensic human ID for 10+ years
  - Casework, missing persons, mass disasters, unidentified remains

- Advantages of mtDNA
  - Exists in high copy number in the cell
    - Successful PCR amplification from very small amount of tissue
    - Can obtain mtDNA information when STR amplification fails
  - Resistant to exonuclease degradation
    - Circular molecule
  - Sequence diversity in the non-coding region (control region, HV1 & HV2)
    - Higher mutation rate than nuclear DNA

- Limitations of mtDNA
  - Single molecule / single marker
    - Does not give as much information as STR profile (13+ markers)
  - Maternally inherited
   - Can only determine familial relationships (not individual)

Mass Spectrometry or Sequencing?

- Simplified workflow vs Sanger Sequencing
  - PLEX-ID: PCR product is analyzed on a fully automated system
  - Reduced cost through <u>savings in labor</u> (wet lab and analysis)
  - Faster turnaround

Example:
One sample mtDNA typing

| **Mass Spec** Cost $180 Time 4 hours | | **Sequencing** Cost $240+ Time 10+ hours |

```
                    DNA Extraction
                         ↓
★ Cleanup PCR  ←  PCR Amplification  →  Cleanup PCR
        ↓                                    ↓
★ Mass Determination                   Sequencing Reaction
        ↓                                    ↓
                                       Sequencing Cleanup
                                             ↓
                                        ★ C. E.
                                             ↓
                                       Sequence Alignment
                                             ↓
  Data Review                            Data Review
```
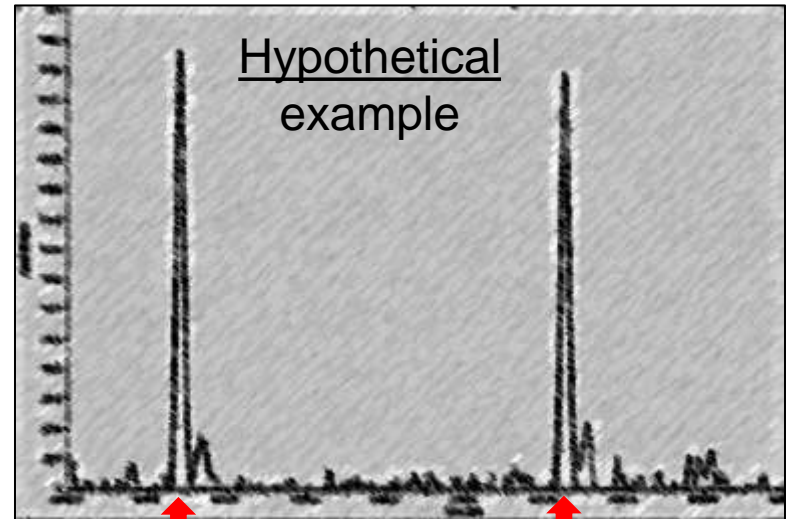
PLEX-ID

★ = Automated step

Sequencing Results are Different From Mass Spectrometry – "Base Composition"

- Sequencing gives an ordered string of bases
- Mass spectrometry only gives a mass measurement
  - We know the masses of nucleotides
  - Base composition of a DNA molecule can be calculated
  - An empirical formula of numbers of A, G, C, and T residues
  - Positional information is lost

C A T C A C G A T G G A T C A C A G

A6 G4 C5 T3

Hypothetical example
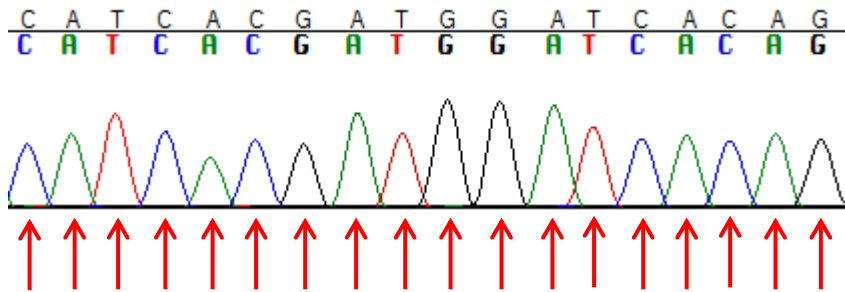
Light strand
5594.6 Daltons
A6 G4 C5 T3

Heavy strand
5617.6 Daltons
A3 G5 C4 T6

PLEX-ID mtDNA 2.0 Coverage

- 24 PCR amplicons ≈ 140 bp each
  - Tiled across the HV1 & HV2 regions of mtDNA
    - Plus additional sites = 1046 bases assayed
  - Eight triplex PCR reactions
    - One column of a 96-well plate = 1 sample

Control Region

HV1		HV2	
16,024 – 16,365		73 – 340	

16,024 ... 16,569/1 ... 576

mtDNA 2.0 Assay

15,924 | 16,428 | 31 | 576

16,251-16,253
Not covered

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16,569/1

mtDNA 2.0 Assay

15,924	16,428	31	576

16,251-16,253
Not covered

Outside C.R.

Not assayed
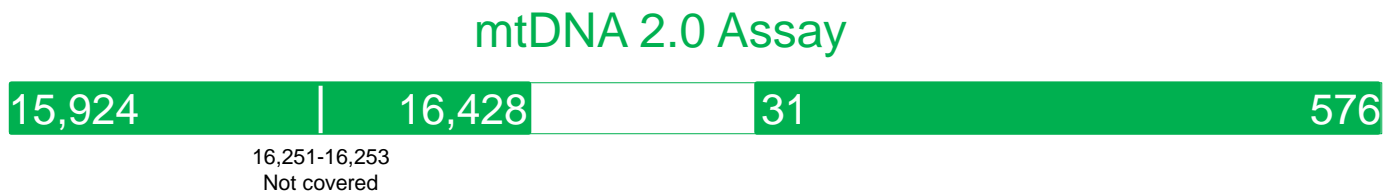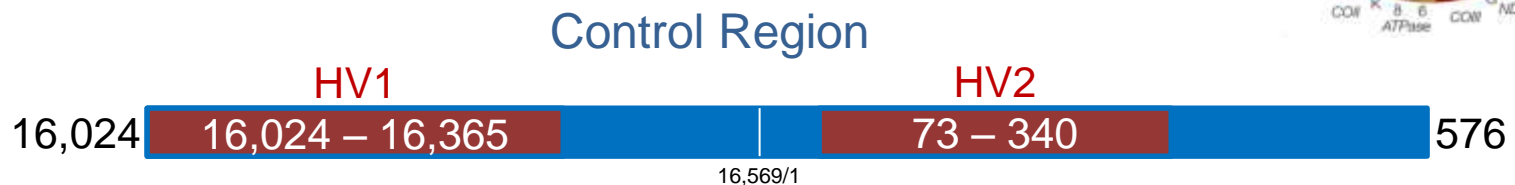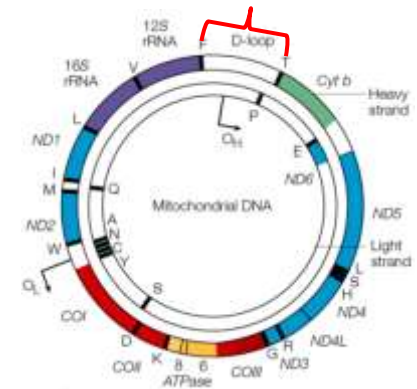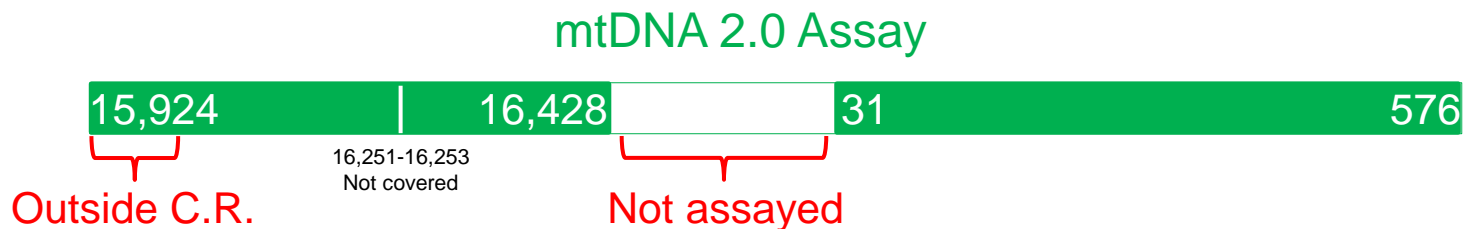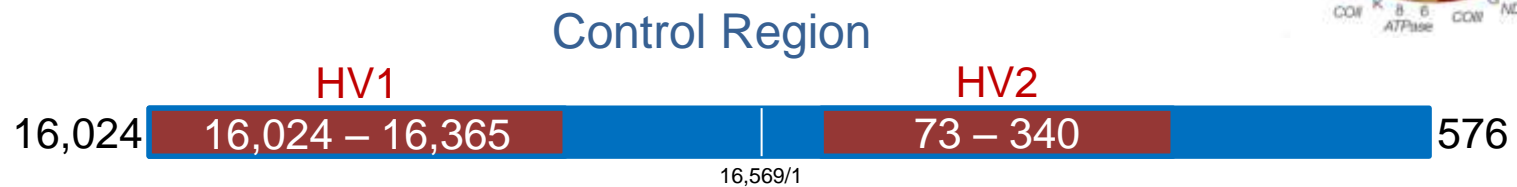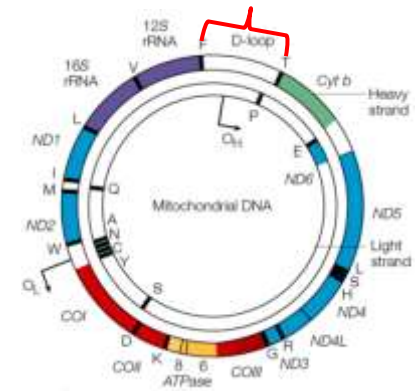
Study Goals

- Evaluate concordance between Sanger sequencing and base composition by PLEX-ID

- Compare "discriminatory power" of results
  - HV1 & HV2 (610 bp) vs. PLEX-ID (1048 bp)
  - Control region (1122 bp) vs. PLEX-ID (1048 bp)

- 711 Sanger sequences converted to base composition
  - 665 sequences generated at AFDIL
    - Saunier *et al. FSI Genetics* 2 (2008) e19-e23
    - Diegoli *et al. FSI Genetics* 4 (2009) e45-e52
  - 46 sequences generated at NIST

Available online at www.sciencedirect.com

ScienceDirect

Forensic Science International: Genetics 2 (2008) e19-e23

Announcement of population data

Mitochondrial control region sequences from a U.S. "Hispanic" population sample

Jessica L. Saunier, Jodi A. Irwin*, Rebecca S. Just, Jennifer O'Callaghan, Thomas J. Parsons[1]

Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, Rockville, MD 20850, United States

Received 9 August 2007; received in revised form 17 October 2007; accepted 6 November 2007

Forensic Science International: Genetics 4 (2009) e45-e52

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Announcement of population data

Mitochondrial control region sequences from an African American population sample

Toni M. Diegoli, Jodi A. Irwin*, Rebecca S. Just, Jessica L. Saunier, Jennifer E. O'Callaghan, Thomas J. Parsons[1]

Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, Rockville, MD 20850, United States

Experimental Method

- Concordance study of 711 samples
  - Four major population groups found in U.S.A.
    - 49 Asian American
    - 260 African American
    - 262 Caucasian
    - 140 Hispanic

- Comparison of 24 amplicons
  - Sanger sequence converted to base composition
  - Do we get the same result as base composition from the PLEX-ID?

- Previous studies
  - Hall *et al.* 2009 *Anal. Chem.* n = 166
  - Warshauer *et al.* 2012 *IJLM* n = 150
  - Howard *et al.* 2013 *FSI Genet*. n = 225
    - T5000 (predecessor to PLEX-ID)


```
pp     coords      Y8_seq                         Y8            Diff
--------------------------------------------------------------------
2901: 15893..16012: NODATA              ---> A48 G17 C25 T30     0
2925: 15937..16041: NODATA              ---> A35 G14 C24 T32     0
2899: 15985..16073: NODATA              ---> A26 G15 C21 T27     0
2898: 16025..16119: A26 G17 C27 T25     ---> A26 G17 C27 T25     0
2897: 16055..16155: A31 G13 C30 T27     ---> A31 G13 C30 T27     0
2896: 16102..16224: A45 G13 C42 T23     ---> A45 G13 C42 T23     0
2895: 16130..16224: A36 G7 C33 T19      ---> A36 G7 C33 T19      0
2893: 16154..16268: A44 G7 C46 T18      ---> A44 G7 C46 T18      0
2892: 16231..16338: A40 G9 C39 T20      ---> A40 G9 C39 T20      0
2891: 16256..16366: A37 G9 C40 T25      ---> A37 G9 C40 T25      0
2890: 16318..16402: A20 G14 C30 T21     ---> A20 G14 C30 T21     0
2889: 16357..16451: A21 G17 C36 T21     ---> A21 G17 C36 T21     0
2902: 5..97:        A19 G24 C24 T26     ---> A19 G24 C24 T26     0
2903: 20..139:      A24 G34 C29 T33     ---> A24 G34 C29 T33     0
2904: 83..187:      A23 G21 C29 T32     ---> A23 G21 C29 T32     0
2905:               48                  ---> A39 G18 C28 T48     0
2906:               40                  --->
2908:               32                  --->
2907:               23                  --->
2923:               20                  --->
2910:               6                   --->
2916:               0                   --->
2912:               6                   --->
2913:               23                  ---> A44 G10 C63 T23     0
Total differences: 0
```

Sequence converted to base composition

Measured base composition

Note: differences in coverage - three amplicons outside control region

Concordance Rate

- PLEX-ID measurements that disagreed with converted Sanger sequence = 0
  - 100% concordant

- Number of incomplete profiles = 5
  - 2 African American (samples AF084 & AF228)
    - Polymorphisms at 89 C, 93 G, 95 C
    - Interfered with PCR reverse primer binding
    - Amplicon 2902 not detected – partial profile (23/24)
  - 2 Asian American (samples 005 & 010) and 1 Hispanic (sample Y27)
    - Extensive C-length heteroplasmy
    - Asian 005 – amplicon 2893 (HV1 C-stretch) not detected – partial profile (23/24)
    - Asian 010 – amplicon 2913 (HV3 C-stretch) not detected – partial profile (23/24)
    - Hispanic Y27 – amplicon 2895 (HV1 C-stretch) not detected – partial profile (23/24)

- Overall concordance rate (full profiles) = 706 / 711 = 99.3%
  - Number of successfully measured amplicons = 17,059
  - Concordance by number of amplicons = 17,059 / 17,064 = 99.97%

Discrimination Capacity

- How does the PLEX-ID base composition method compare with sequencing in differentiating mtDNA types?

- 706 samples with complete profiles in PLEX-ID database
  - Search database "against self" for identical profiles
  - Assistance from Tom Hall at Abbott in creating algorithm

- Same 706 samples' control region sequences
  - Create multiple sequence alignment
  - Identify groups with common sequences
    - Limited to just HV1 & HV2
    - Full control region

- Results form "common haplotype groups" of identical mito types

- Discrimination capacity (DC)
  = number of haplotypes observed / population size

Unique Mito Types

n = 706

# of Members in Group	Mass spec. Plex-ID	Sequence HV1 & 2	Sequence 16024 - 576
1	522	499	549
2	36	32	31
3	12	14	12
4	4	4	4
5	5	6	4
6	1	4	2
7	1	1	-
8	-	1	-
9	1	-	-
10	-	-	-
11	-	-	1
12	-	-	-
13	1	-	-
14	-	-	-
15	-	-	-
16	-	1	-
% unique	73.9%	70.7%	77.8%
DC	0.83	0.80	0.85
Coverage	1048 bp	610 bp	1122 bp

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Left list:

CN_Y6
CN101
CN242
CN246
CN248
CN258
CN259
CN263
CN266
CN275
CN281
CN282
CN284

Right list:

CN_Y6
CN242
CN246
CN258
CN259
CN263
CN266
CN275
CN281
CN282
CN284
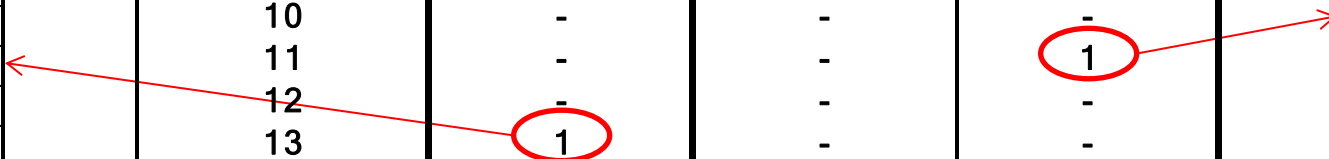
- Groups of samples with common haplotypes

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1	522	499	549
2	36	32	31

Comparison of unique mito haplotypes
PLEX-ID vs. HV1 / HV2 sequencing = 4.5%
PLEX-ID vs. full control region sequencing = -5.0%

8	-	1	-
9	1	-	-
10	-	-	-
11	-	-	1
12	-	-	-
13	1	-	-
14	-	-	-
15	-	-	-
16	-	1	-
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9	1	-	-
10	-	-	-
11	-	-	1
12	-	-	-
13	1	-	-
14	-	-	-
15	-	-	-
16	-	1	-
% unique	73.9%	70.7%	77.8%
DC	0.83	0.80	0.85
Coverage	1048 bp	610 bp	1122 bp

- **Discrimination capacity (DC) = h/n**
  h = number of haplotypes observed
  n = number of samples in population

Unique Mito Types

n = 706

# of Members in Group	Mass spec. Plex-ID	Sequence HV1 & 2	Sequence 16024 - 576
1	522	499	549
2	36	32	31
3	12	14	12
4	4	4	4

Comparison of discrimination capacity
PLEX-ID vs. HV1 / HV2 sequencing = 3.6%
PLEX-ID vs. full control region sequencing = -2.3%

# of Members in Group	Mass spec. Plex-ID	Sequence HV1 & 2	Sequence 16024 - 576
10	-	-	-
11	-	-	1
12	-	-	-
13	1	-	-
14	-	-	-
15	-	-	-
16	-	1	-
% unique	73.9%	70.7%	77.8%
DC	0.83	0.80	0.85
Coverage	1048 bp	610 bp	1122 bp

Performance in Populations

- Haplotype groups separated by population
  - Is there population bias?

# of Members in Group	Asian American n = 47			African American n = 258			Caucasian n = 262			Hispanic n = 139		
	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.
1	47	47	47	192	187	198	182	170	196	101	95	108
2	-	-	-	13	12	16	9	11	7	14	9	8
3	-	-	-	6	6	8	4	5	2	2	3	2
4	-	-	-	2	1	-	2	2	4	-	1	-
5	-	-	-	1	1	1	4	4	3	-	1	-
6	-	-	-	-	2	-	-	1	1	1	1	1
7	-	-	-	-	-	-	1	1	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-
9	-	-	-	1	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	1	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	1	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	1	-	-	-	-
% unique	100%	100%	100%	74%	72%	77%	69%	65%	75%	73%	68%	78%
DC	1.00	1.00	1.00	0.83	0.81	0.86	0.77	0.74	0.82	0.85	0.79	0.86

Performance in Populations

- ## Small sample size for Asian American population
  - ### All unique mito types

# of Members in Group	Asian American n = 47			African American n = 258			Caucasian n = 262			Hispanic n = 139		
	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.
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4	-	-	-	2	1	-	2	2	4	-	1	-
5	-	-	-	1	1	1	4	4	3	-	1	-
6	-	-	-	-	2	-	-	1	1	1	1	1
7	-	-	-	-	-	-	1	1	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-
9	-	-	-	1	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	1	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	1	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	1	-	-	-	-
% unique	100%	100%	100%	74%	72%	77%	69%	65%	75%	73%	68%	78%
DC	1.00	1.00	1.00	0.83	0.81	0.86	0.77	0.74	0.82	0.85	0.79	0.86

Performance in Populations

- PLEX-ID performed similarly across 3 ethnic groups
  - African American, Caucasian, and Hispanic

# of Members in Group	Asian American n = 47			African American n = 258			Caucasian n = 262			Hispanic n = 139		
	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.
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3	-	-	-	6	6	8	4	5	2	2	3	2
4	-	-	-	2	1	-	2	2	4	-	1	-
5	-	-	-	1	1	1	4	4	3	-	1	-
6	-	-	-	-	2	-	-	1	1	1	1	1
7	-	-	-	-	-	-	1	1	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-
9	-	-	-	1	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	1	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	1	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	1	-	-	-	-
% unique	100%	100%	100%	74%	72%	77%	69%	65%	75%	73%	68%	78%
DC	1.00	1.00	1.00	0.83	0.81	0.86	0.77	0.74	0.82	0.85	0.79	0.86

Performance in Populations

- ## Sequencing HV1 & HV2
  - ### Similar trend, no significant difference in performance

| # of Members in Group	Asian American n = 47			African American n = 258			Caucasian n = 262			Hispanic n = 139		
	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.
1	47	47	47	192	187	198	182	170	196	101	95	108
2	-	-	-	13	12	16	9	11	7	14	9	8
3	-	-	-	6	6	8	4	5	2	2	3	2
4	-	-	-	2	1	-	2	2	4	-	1	-
5	-	-	-	1	1	1	4	4	3	-	1	-
6	-	-	-	-	2	-	-	1	1	1	1	1
7	-	-	-	-	-	-	1	1	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-
9	1	-	-	1	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	1	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	1	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	1	-	-	-	-
% unique	100%	100%	100%	74%	72%	77%	69%	65%	75%	73%	68%	78%
DC	1.00	1.00	1.00	0.83	0.81	0.86	0.77	0.74	0.82	0.85	0.79	0.86

Performance in Populations

- ## Sequencing full control region
  - ### Similar trend, no significant difference in performance

# of Members in Group	Asian American n = 47			African American n = 258			Caucasian n = 262			Hispanic n = 139		
	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.	PLEX	HV1/2	Full C.R.
1	47	47	47	192	187	198	182	170	196	101	95	108
2	-	-	-	13	12	16	9	11	7	14	9	8
3	-	-	-	6	6	8	4	5	2	2	3	2
4	-	-	-	2	1	-	2	2	4	-	1	-
5	-	-	-	1	1	1	4	4	3	-	1	-
6	-	-	-	-	2	-	-	1	1	1	1	1
7	-	-	-	-	-	-	1	1	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-
9	-	-	-	1	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	1	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	1	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	1	-	-	-	-
% unique	100%	100%	100%	74%	72%	77%	69%	65%	75%	73%	68%	78%
DC	1.00	1.00	1.00	0.83	0.81	0.86	0.77	0.74	0.82	0.85	0.79	0.86

Conclusions

- **Significant savings in labor & turnaround time**
  - Useful for screening large numbers of samples

- **Excellent fidelity with sequencing results**

- **PLEX-ID mtDNA 2.0 is more discriminating than HV1 & HV2 sequence**
  - 3.6% more discrimination capacity

- **Full control region sequencing contains the most information**
  - 2.6% more discrimination capacity than PLEX-ID

- **No population bias identified**
  - Limited data on Asian population

- **Potential improvement**
  - More coverage of control region
  - 16,519 C present in ≈ 50% of 711 NIST population samples
  - Could improve discrimination capacity
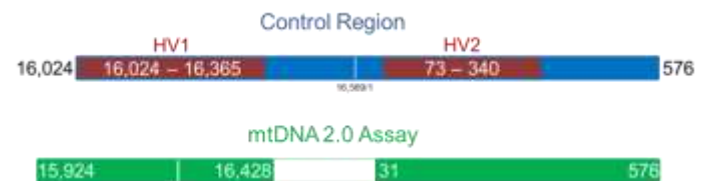
Acknowledgments

Contact Info:
Kevin.Kiesler@nist.gov
301-975-4306

Questions?

My contact info:
email: Kevin.Kiesler@NIST
Phone: (301) 975-4306