FORENSICS @ NIST
November 28-30, 2012 • #NISTForensics

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

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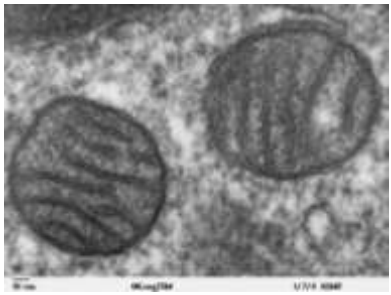
Forensics@NIST 2012 Meeting
Gaithersburg, MD
November 28, 2012

Outline

- Mitochondrial DNA typing
- Why use Mass Spectrometry?
- Abbott / Ibis Biosciences PLEX-ID Instrument
- PLEX-ID mtDNA 2.0 Assay
- Evaluation Experiments
- Future directions

Mitochondrial DNA

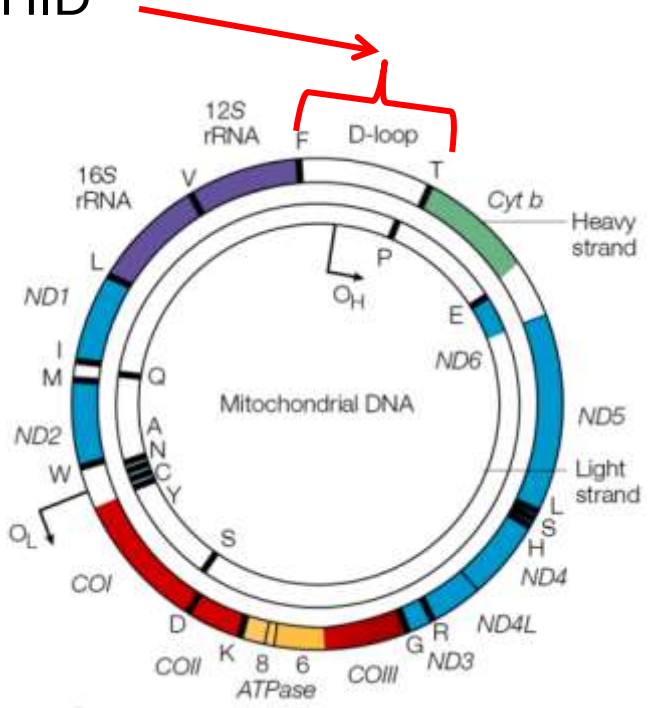


- Mitochondria are organelles within cells
  - Produce energy via the Krebs Cycle
- Separate genome from the nucleus
  - ≈ 16,569 bp
- Human cells have hundreds of mitochondria
- Each mitochondrion has between 2 – 10 genome copies
  - One cell = 2 nuclear genome copies ≈ 1000 mtDNA copies
- High copy number of mtDNA can be useful for PCR amplification
  - Sometimes quantity of forensic evidence is a limitation
  - Trace evidence (hair & bone)
  - When nuclear STR profile fails, can often obtain mtDNA results

http://remf.dartmouth.edu/images/mammalianLungTEM/source/8.html

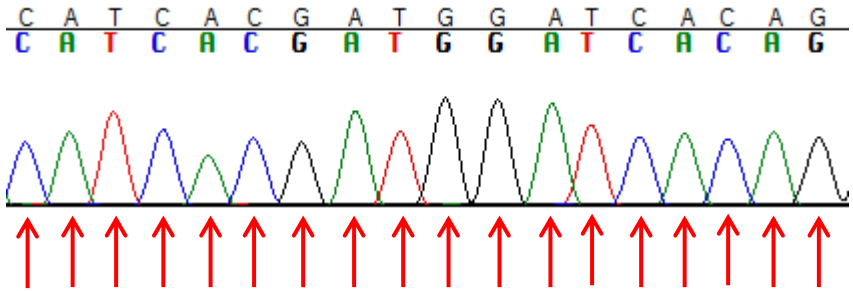
mtDNA Genotyping for Human I.D.

- Mutations in mtDNA occur naturally & accumulate over generations
  - Mutations allow for differentiating people based on DNA sequence
  - mtDNA is passed on only from mothers to children (maternal lineage)
  - Can only be used for lineage identification, not individual I.D.
    - Brothers and sisters (& some cousins) will have the same mtDNA sequence
- Non-coding “hypervariable region” is used for HID
  - Nucleotides 16,024 – 574
  - Approximately 1122 bp
- Assayed by Sanger DNA sequencing
  - Gold standard for accuracy
  - Fluorescent dye terminator bases
  - Capillary electrophoresis



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 inferred
  - An empirical formula of numbers of A, G, C, and T residues
  - Positional information is lost

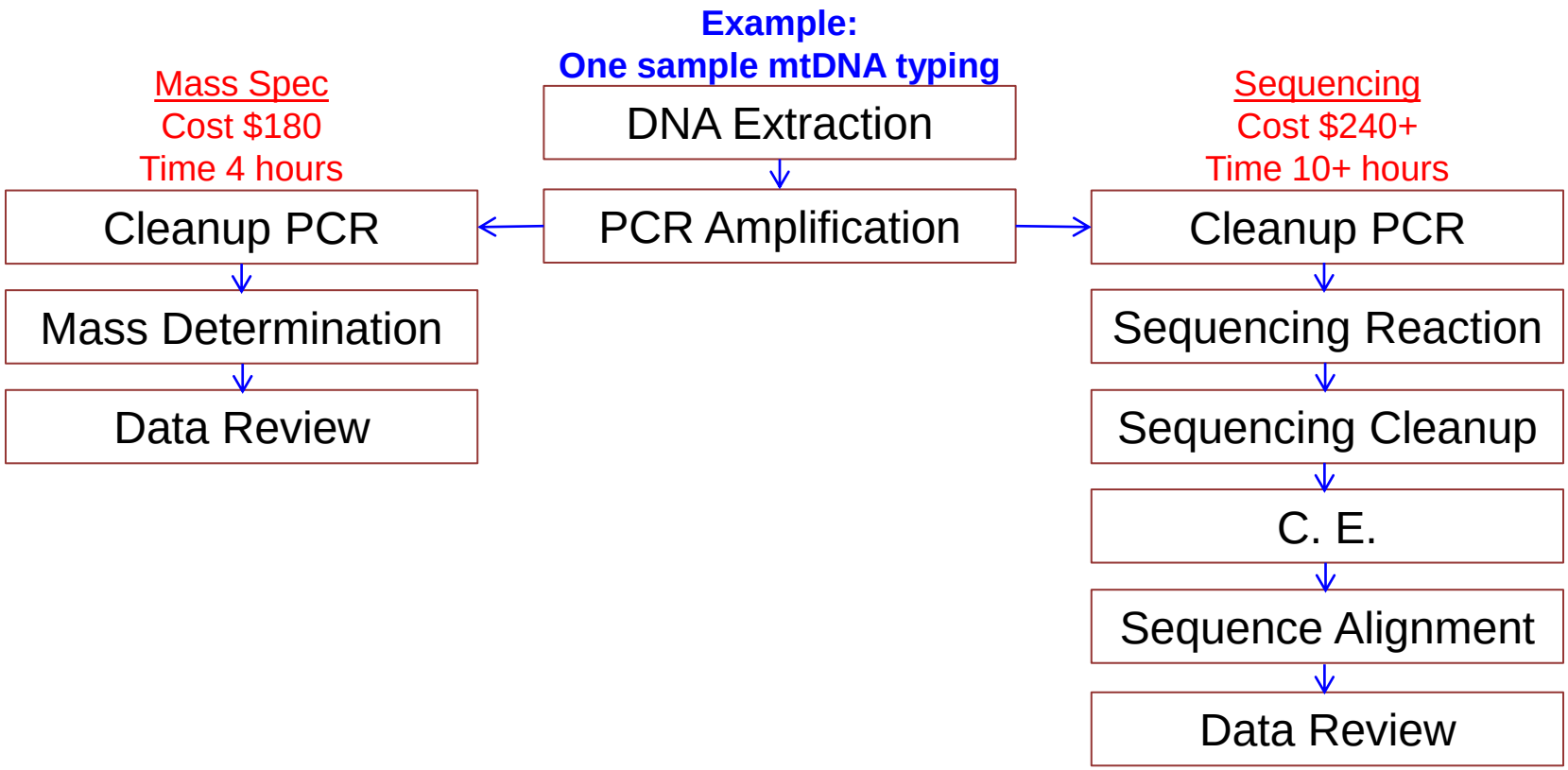


A6 G4 C5 T3

- Base composition result is almost equally as informative as sequence

Why Use Mass Spectrometry?

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



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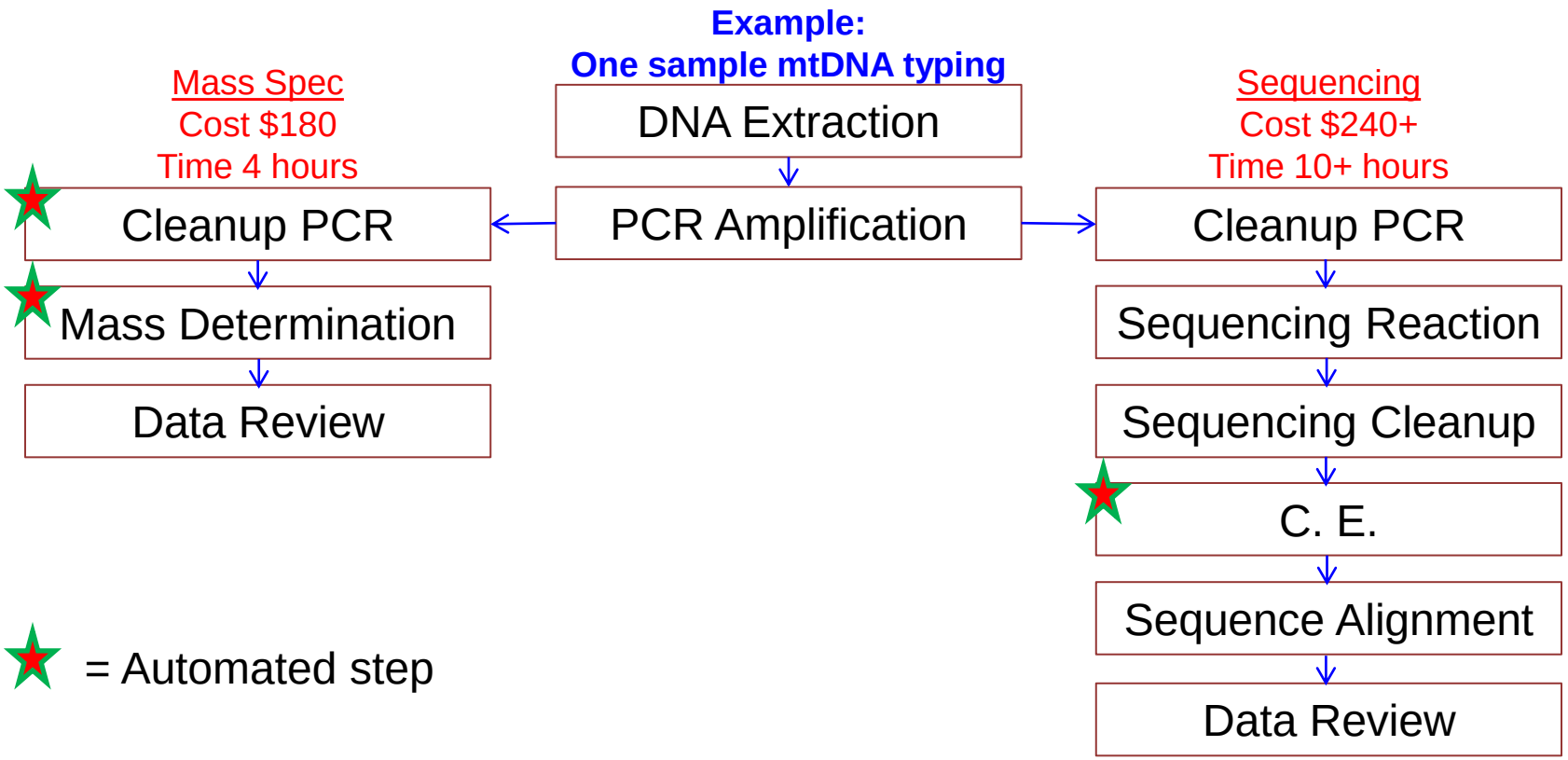
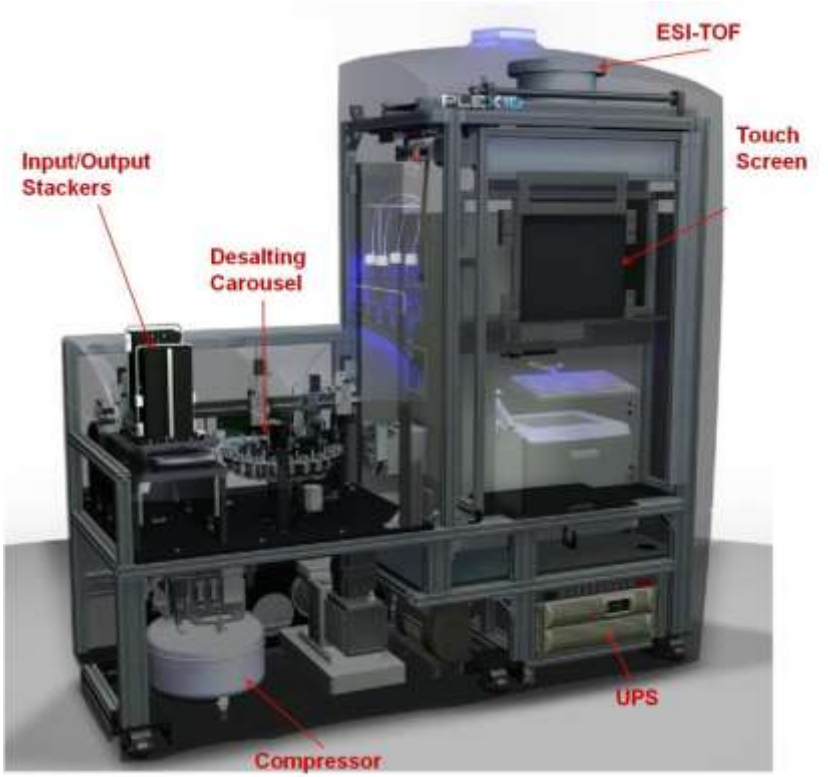


The PLEX-ID Instrument

- Mass spectrometer designed solely for analysis of DNA (PCR)
- Fully automated
  - Plate stacker holds up to 15 PCR plates
  - Desalting by magnetic bead cleanup
    - Cleanup reagents stored onboard
  - Fluidics system handles all sample transfers including injection into mass spectrometer
- Data analysis on separate computer

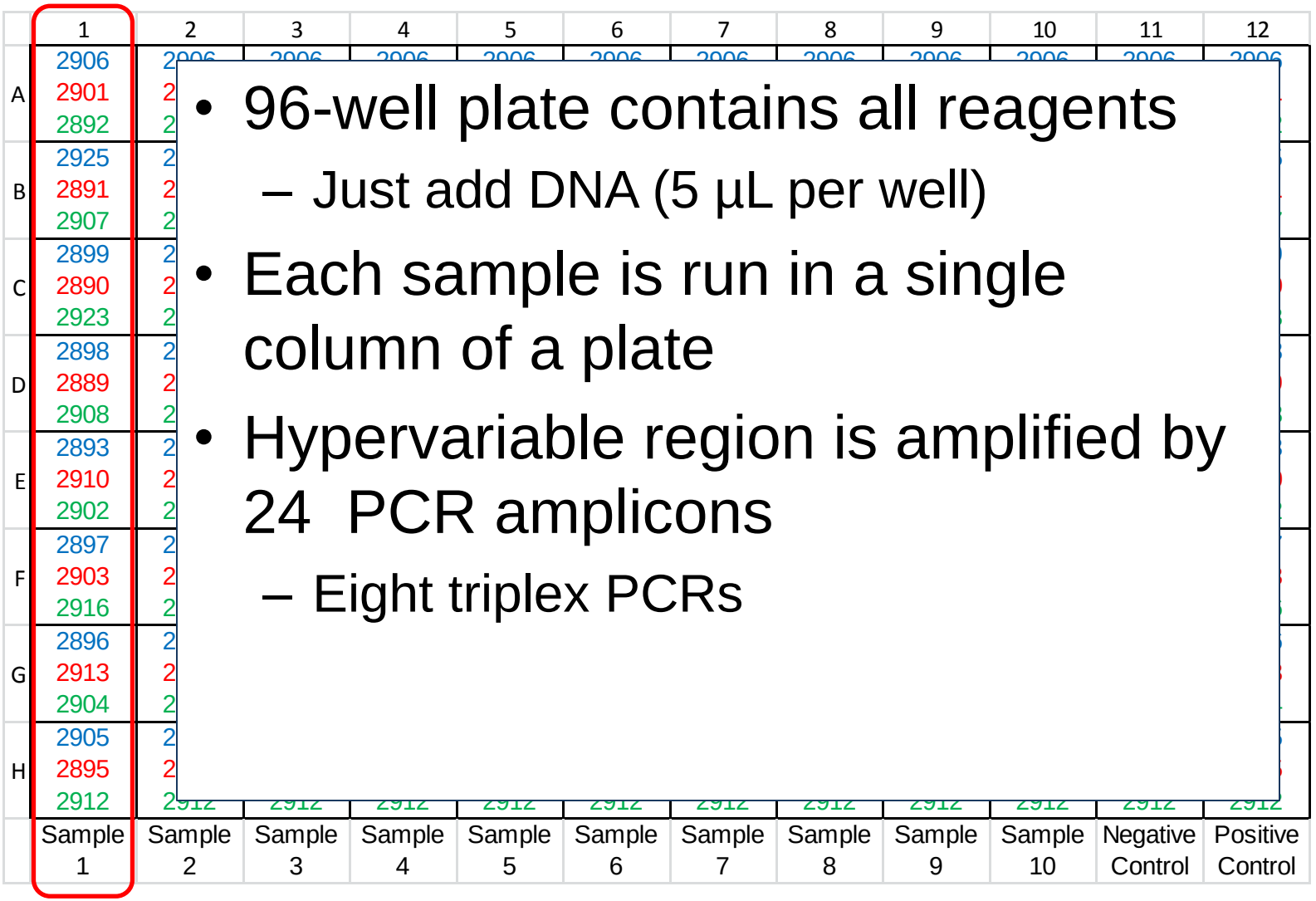


Electrospray Ionization Time-of-Flight Analysis

- Soft ionization method
- Does not fragment molecules
- DNA strands of PCR product are dissociated on injection
- DNA molecular masses are measured
  - Forward and reverse strands measured separately
- Mass is converted to a result by comparing to reference database of known masses
- Results:
  - mtDNA base composition profile
  - STR profile
  - SNP genotypes

PLEX-ID

mtDNA 2.0 Assay Plate Layout

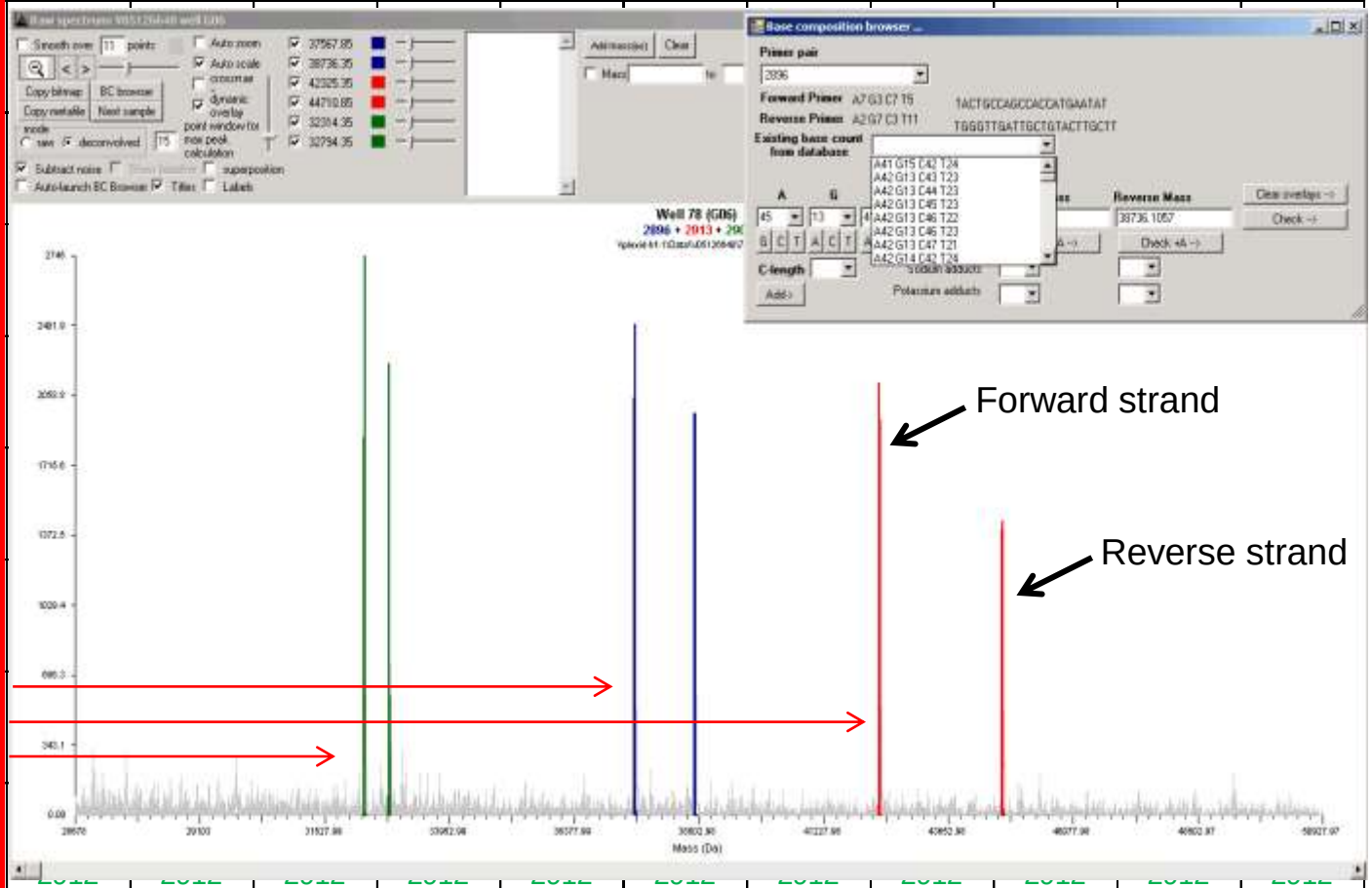


- 96-well plate contains all reagents
  - Just add DNA (5 µL per well)
- Each sample is run in a single column of a plate
- Hypervariable region is amplified by 24 PCR amplicons
  - Eight triplex PCRs

Applied Genetics

mtDNA 2.0 Assay - Result

	1	2	3	4	5	6	7	8	9	10	11	12
A	2906 2901 2892											
B	2925 2891 2907											
C	2899 2890 2923											
D	2898 2889 2908											
E	2893 2910 2902											
F	2897 2903 2916											
G	2896 2913 2904											
H	2905 2895 2912											
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Negative Control	Positive Control



Evaluation 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

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

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Abbott Product Recall

- The PLEX-ID system is being voluntarily recalled
  - Due to reliability issues reported by clinical users
    - Clinical labs cannot tolerate down time
  - Instruments are being removed from the field
  - New more robust instrument under development
    - Estimated to be several years to re-release

- Our experiments support the viability of mass spectrometry technology for DNA based human identification

Future Directions – New Technology

- Ultra high throughput sequencing
  - For deep sequencing of entire mtDNA genome
  - Can generate hundreds of millions of bases of sequence
  - Run completes in 5 hours

- Trained on Life Technologies instrument
  - Ion Torrent Personal Genome Machine (PGM)
  - Bench-top scale next-generation sequencer

PGM

Pilot Studies With Next-Gen Sequencing

- Mitochondrial sequencing standards
  - SRM 2392 and 2392-I
  - Sequenced these three mtDNA genomes on one PGM run
  - 150 million aligned bases
  - Average coverage depth 1427.5 x
  - Now comparing to certified sequence (Sanger method)

SRM 2392

SRM 2392-I

NIST Applied Genetics

Acknowledgments

NIST Team for This Work

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Funding from the **FBI Biometrics Center of Excellence** 'Forensic DNA Typing as a Biometric Tool'