DEVELOPMENT OF THREE MITOCHONDRIAL DNA STANDARD REFERENCE MATERIALS

Barbara C. Levin

National Institute of Standards and Technology

Developing Quality Control Materials for Genetic Testing
Centers for Disease Control and Prevention
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NIST mtDNA STANDARD REFERENCE MATERIALS

SRM 2392: Human mtDNA Amplification and Sequencing Standard # 1; Contains two normal DNA templates: CHR & 9947A; Available since 1999.

SRM 2392-I: Human mtDNA Amplification and Sequencing Standard # 2; Contains HL-60 DNA: a promyelocytic cell line from peripheral blood leukocytes from a 36 year old Caucasian female with acute promyelocytic leukemia. Available since May 2003.

SRM 2394: Heteroplasmic Human mtDNA containing various mixtures of CHR and 9947A. Available 9/2004

Other DNA completely sequenced for comparison:

GM03798 (normal lymphoblastoid cell line)

GM10742A (lymphoblast cell line from patient with Leber Hereditary Optic Neuropathy (LHON).

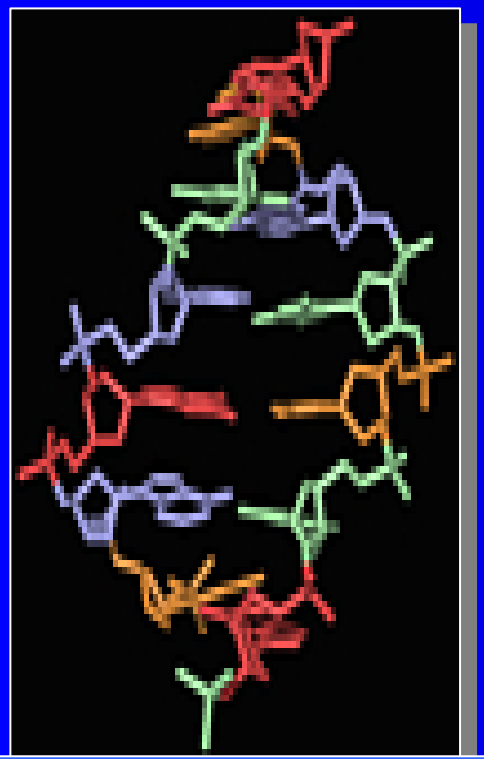
DNA Advisory Board
Quality Assurance Standards
for Forensic DNA Testing Laboratories

Signed by FBI Director on July 15, 1998

STANDARD 9.5

The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

mtDNA Standards 2392 & 2392-I



Includes extracted DNA and all information for performing:

- PCR amplification process
- cycle sequencing steps
- data analysis to determine DNA sequence
- materials to assess accuracy of results

Sequence information for 58 sets of unique primers are included to allow any area or all the mtDNA to be amplified & sequenced

SRMs provide necessary *quality control* for DNA sequence data used to determine genetic predisposition to certain diseases

False Negative	*False Positive*
Incorrect Sense of Security	Loss of Insurance and/or Unnecessary Surgery

Cambridge Reference Sequence nucleotide differences in the five DNA templates included in SRM 2392 and SRM 2392-I

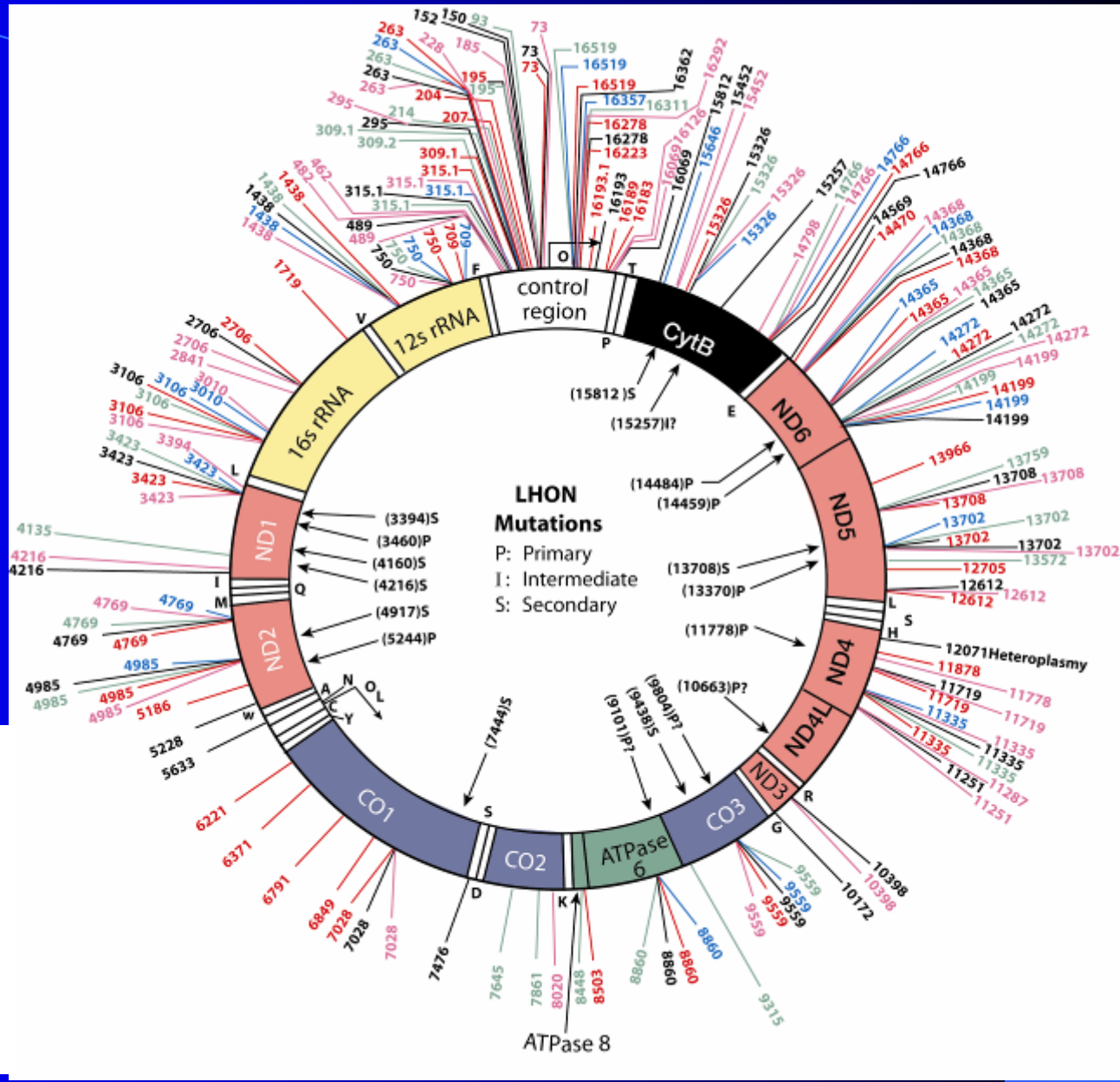
CHR: red

9947A:green

HL-60: black

GM03798:blue

GM10742A:purple



HUMAN HETEROPLASMIC mtDNA SRM 2394

OBJECTIVES:

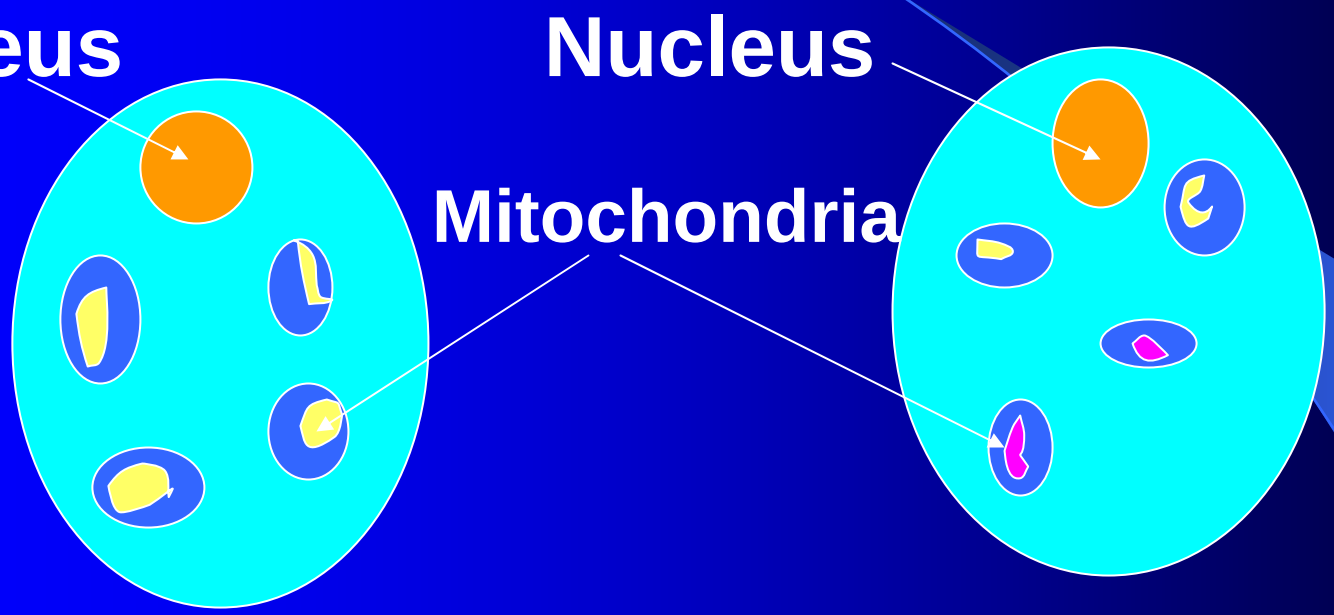
To determine the sensitivity of one's detection techniques for low frequency mutations, SNPs or heteroplasmic DNA.

To help develop tools to enhance the level of detection.

CONTENTS:

Different percentages (1, 2.5, 5, 10, 20, 30, 40 and 50%) of a single nucleotide polymorphism (SNP) heteroplasmic site in a 285 bp PCR product.

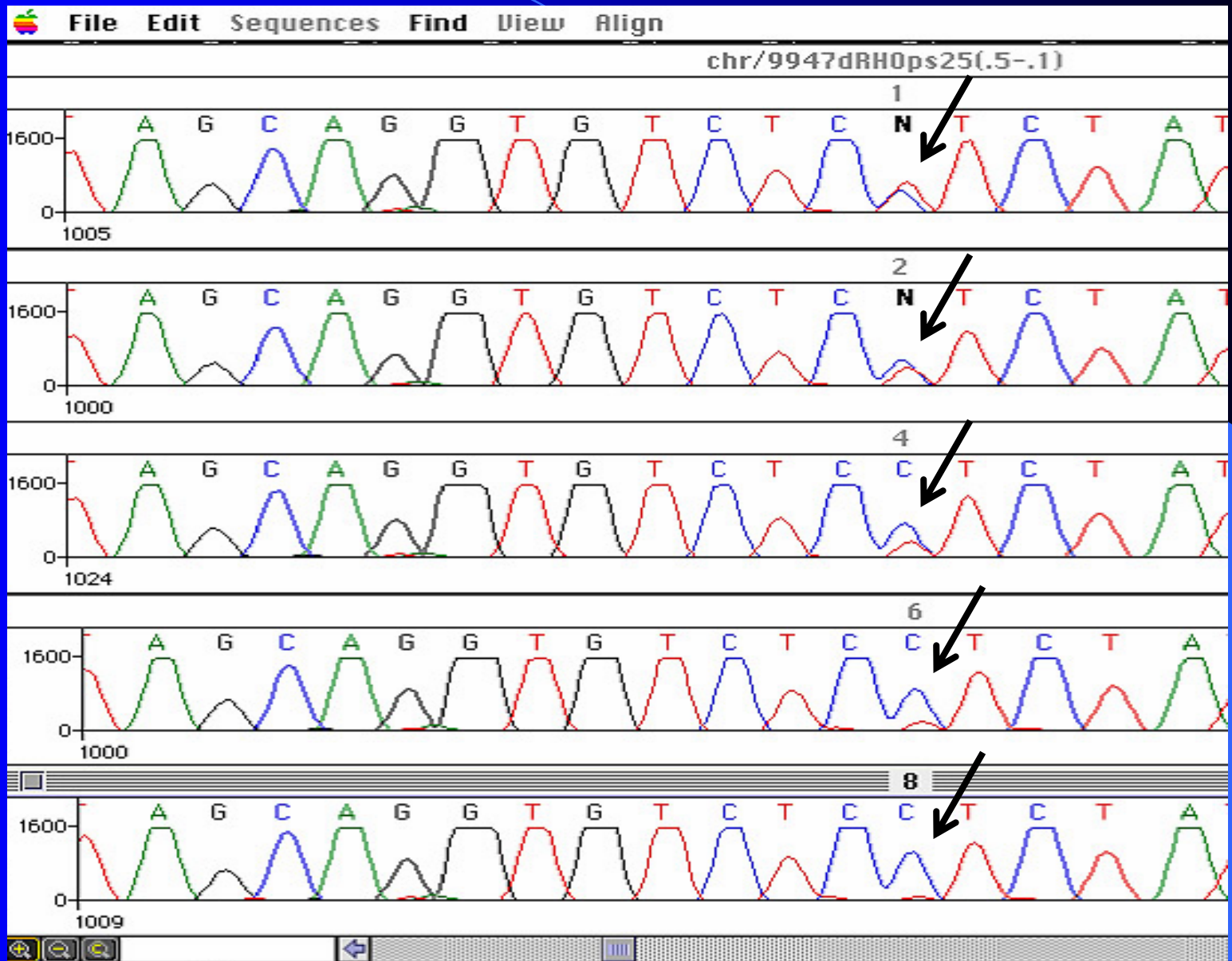
Homoplasmy Heteroplasmy



Normal Mitochondrial DNA (yellow)

50% Mutant Mitochondrial DNA (purple)

Heteroplasmy at Various Levels



50:50

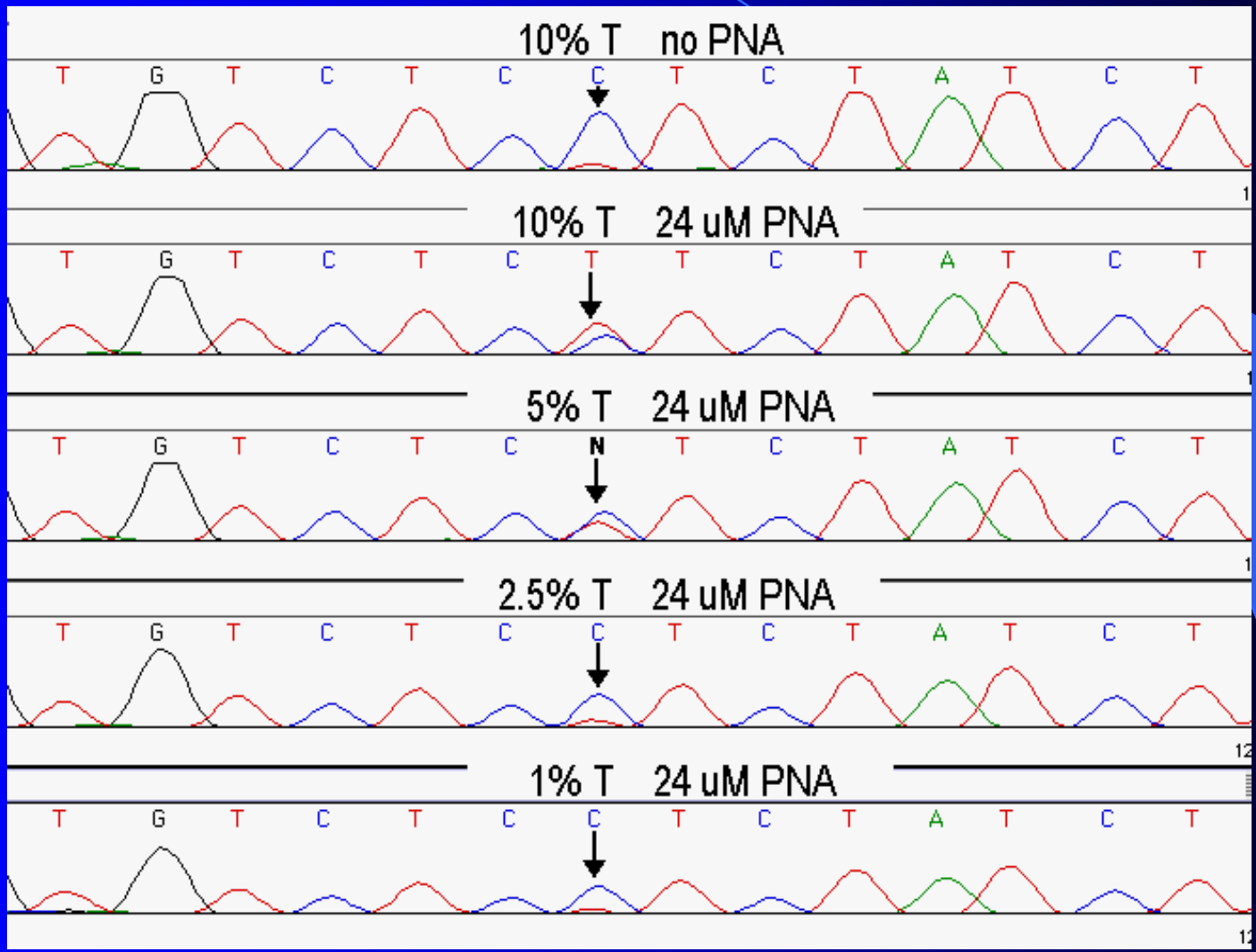
60:40

70:30

80:20

90:10

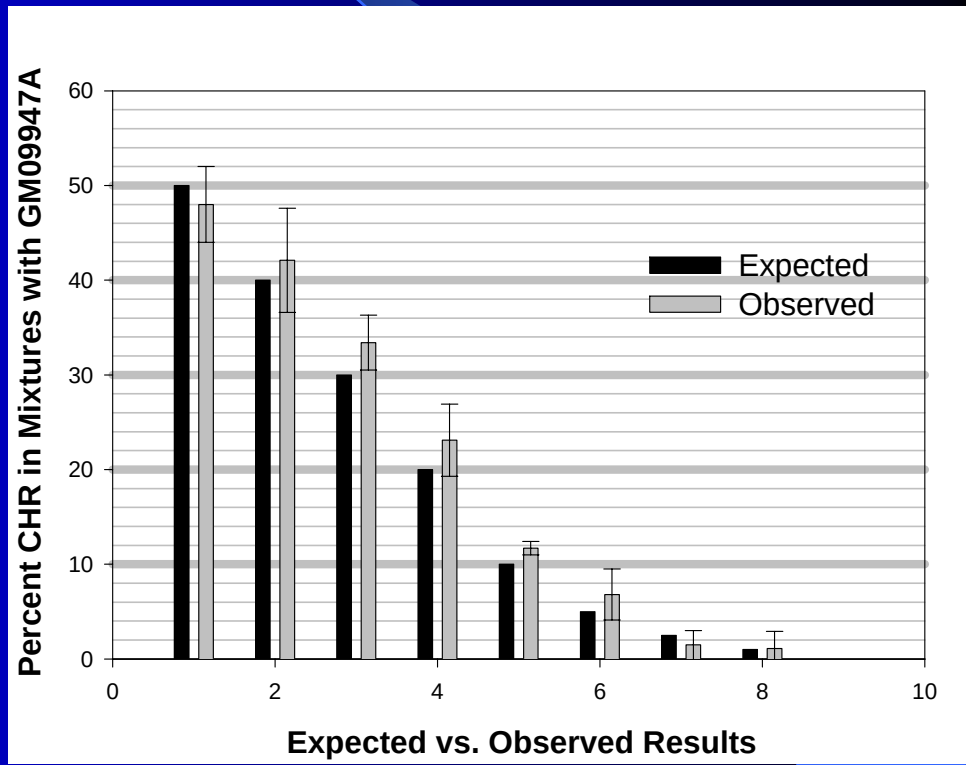
IMPROVED HETEROPLASMY DETECTION USING PEPTIDE NUCLEIC ACIDS



HETEROPLASMY DETECTION OF SRM 2394 USING LUMINEX100 SYSTEM

Detection to low levels - 1%

- Simulated heteroplasmy: 50, 40, 30, 20, 10, 5, 2.5 and 1% mixtures
- Sequencing sensitivity required 20% to be detected above noise.
- Luminex beads designed w/ capture oligos:
  5' GGTGTCTCCTCTATCTTAG
  5' GGTGTCTCTTCTATCTTAG
- Mutant was detected down to 1%



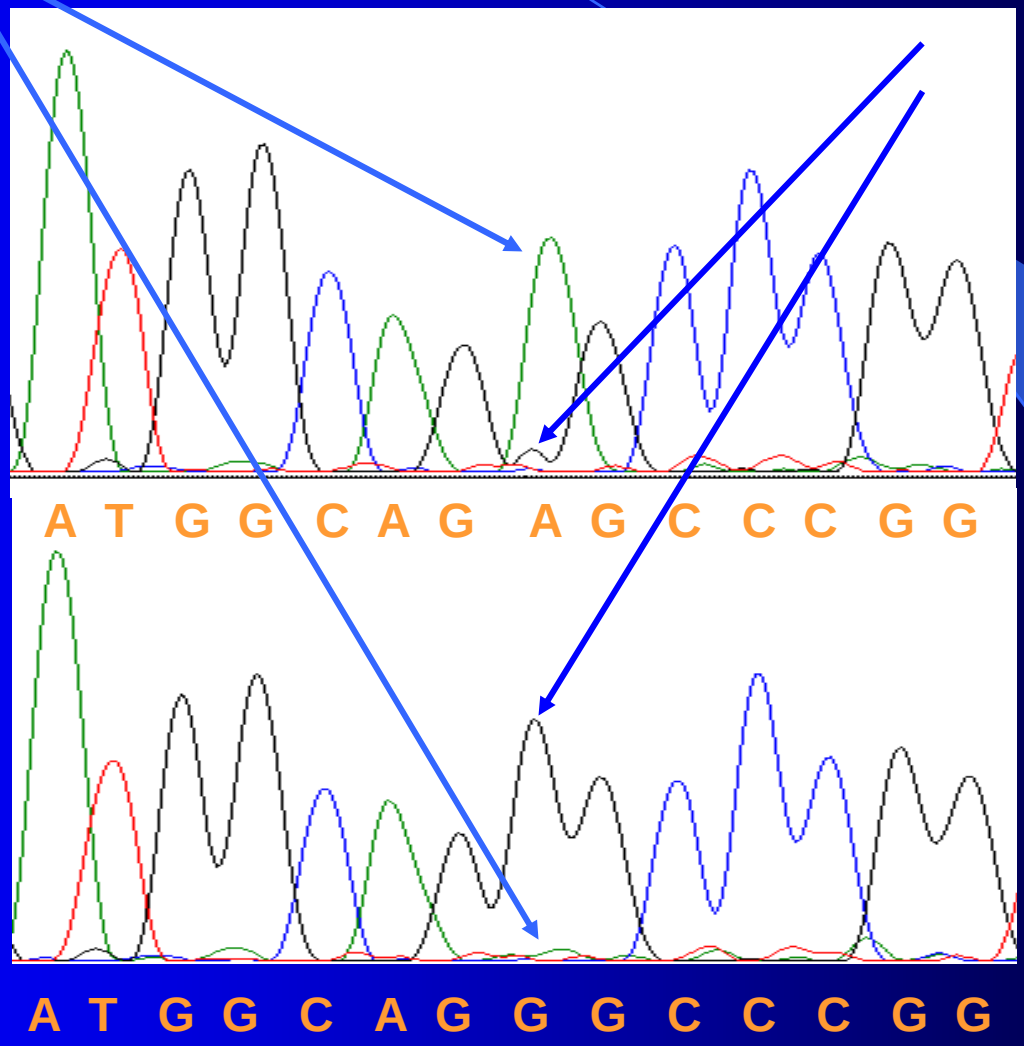
DETECTION OF THE MELAS MUTATION: A HUMAN MITOCHONDRIAL DNA DISEASE

Major Heteroplasmy 3243 A

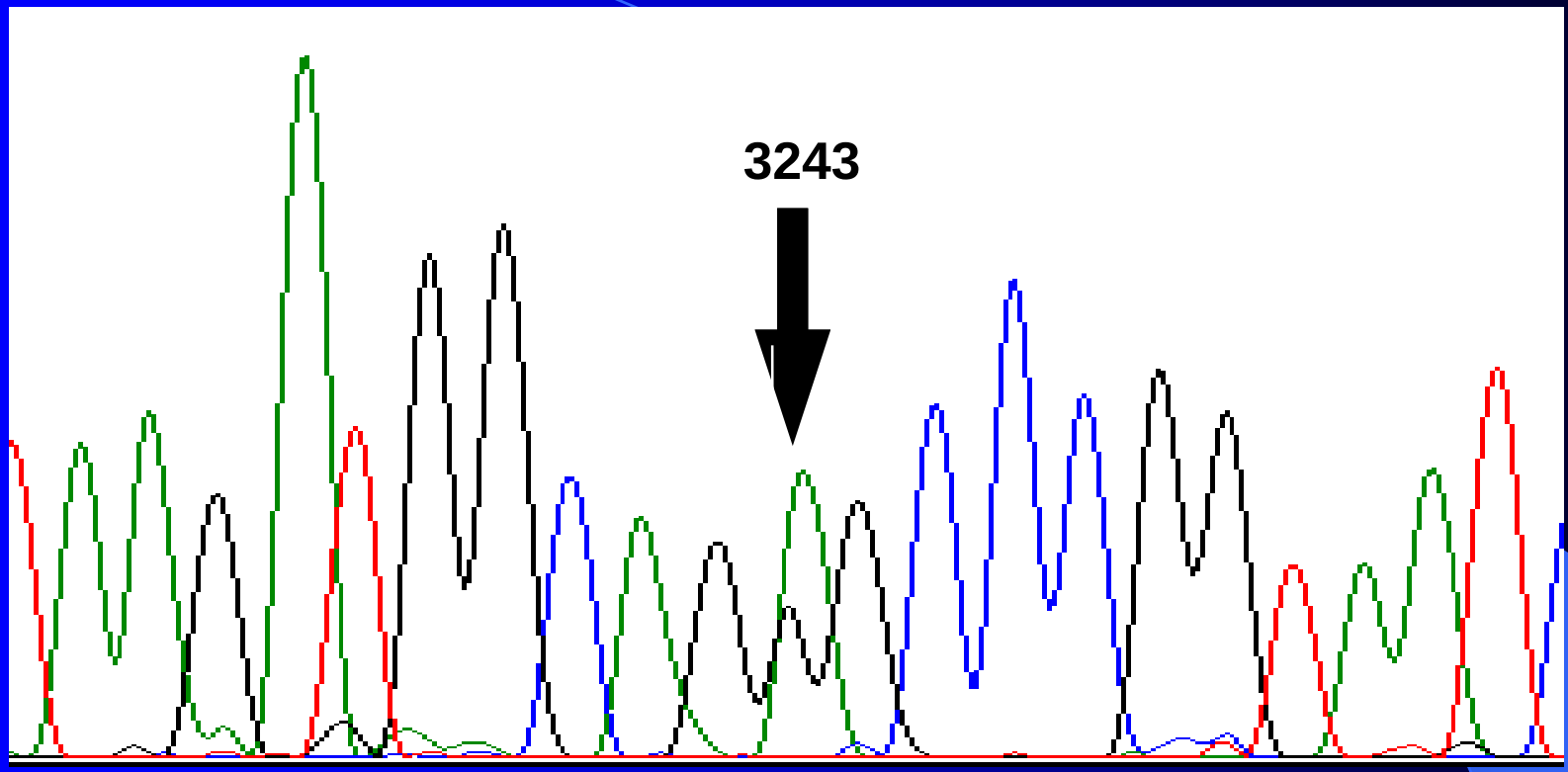
Minor Heteroplasmy 3243 G

PCR Product of Patient Sample (No PNA)

PCR Product with 2μM PNA



PNA Limit of Detection ~ 0.1%



The MELAS sample from patient 7 (1% heteroplasmy) was diluted with wild-type DNA to provide samples with lower levels of mutation. The electropherogram of the PCR product of a 10-fold dilution with 2 μM PNA clearly shows the presence of the mutation, though it is no longer the dominant component.

WEB SITES & E-MAIL

- **Standard Reference Materials Program**
  http://www.nist.gov/srm
- **e-mail: srminfo@nist.gov**
  barbara.levin@nist.gov
- **Interesting WEB sites:**
  http://www.mitomap.org/

http://www.cstl.nist.gov/biotech/strbase/mitoanalyzer.html

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- Koren A. Holland, IPA, Gettysburg College

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