



Technology Transition Workshop



Introduction to Mitochondrial DNA Analysis

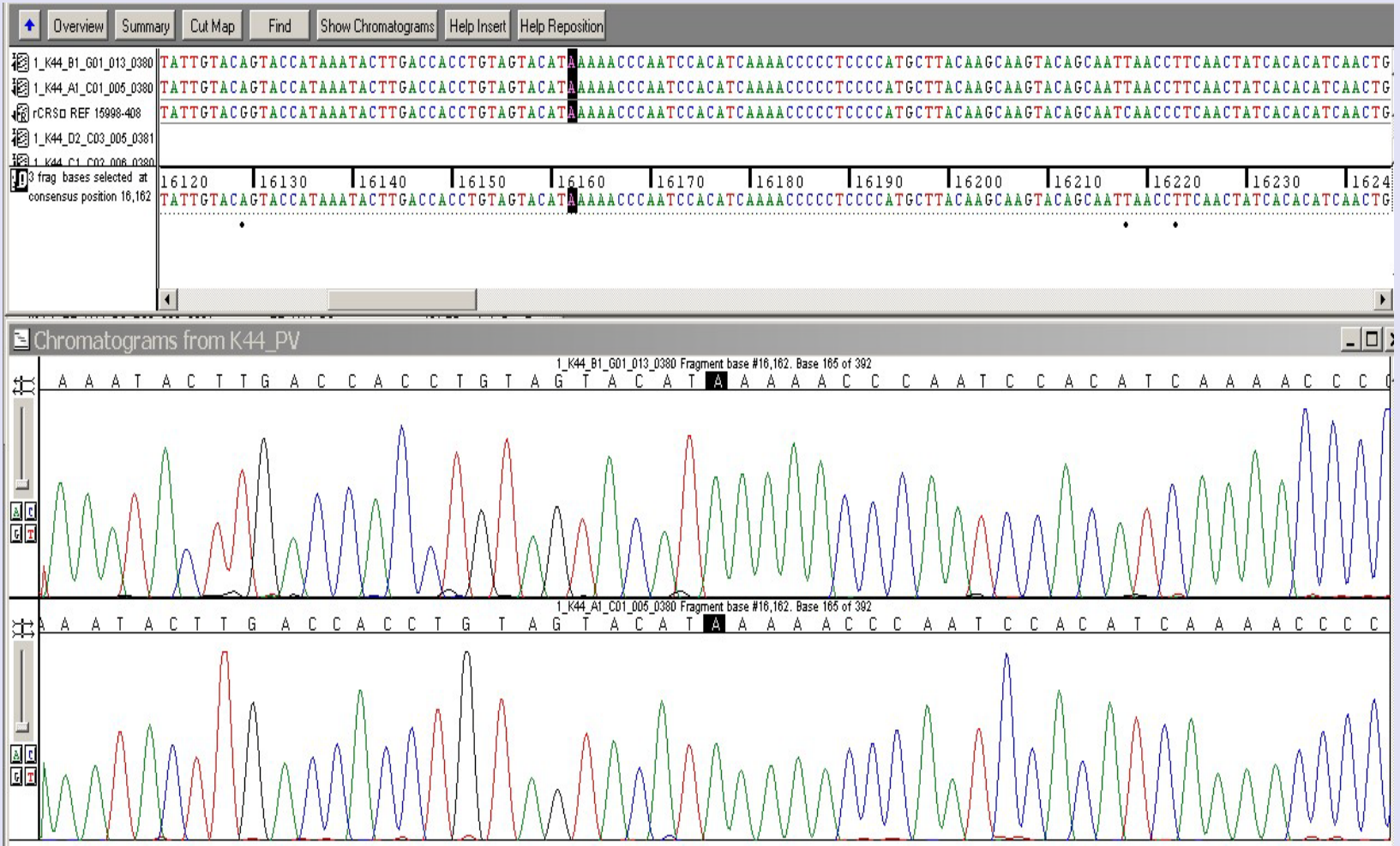
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Deoxyribonucleic Acid

- Nuclear DNA (nucDNA)
 - Biparental inheritance
 - Can achieve unique identity
 - Chromosomal structure
 - One genome/cell
 - Short Tandem Repeats (STR)

- Mitochondrial DNA (mtDNA)
 - Uniparental inheritance along maternal lineage
 - Common between mother & child, siblings, other maternal relatives
 - Circular structure
 - 100's to 1000's copies/cell
 - Hair, teeth, bones, limited or compromised remains or tissues
 - Sequence of bases

mtDNA Sequence Analysis



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L.D. McCurdy

Mitochondrial DNA (mtDNA)

- ~16,569 bases
- Control region
 - Site of origin of replication & transcription
 - High variation (HV1, HV2, HV3)
- Coding region
 - rRNA, tRNA, oxphos proteins
 - Variation scattered
- High copy number
- Polycistronic message
- Different genetic code
 - nucMet=ATG
 - mtMet=ATA
- 10x higher mutation rate
 - Polymerase γ
 - Compromised repair mechanisms
- Maternal inheritance
- Single locus
 - Bases are not independent

History of mtDNA

- mtDNA discovered in early 1960's
 - Luft's Disease
- Complete sequence published in 1981
 - Revised in 1991
- **Medical scientists:**
 - Diseases linked to mtDNA mutations
- **Evolutionary biologists:**
 - Evolution of humans & other species
- **Molecular Anthropologists:**
 - Migration of people(s) throughout world

History of mtDNA

- Court testimony
 - August 1996
 - Tennessee vs Paul William Ware
 - International
 - Federal, State, & Local jurisdiction and appellate decisions
- Unknown Soldier (Michael Blassie)
- Tsar Nicholas II & Romanov family
- Anna Anderson Manahan
- Jesse James
- September 11, 2001

Forensic mtDNA Analysis

- Sample size
 - 2mm hair
- Sample type
 - Hairs, bones, teeth
- Sample condition
 - Degraded?
 - Mixture?
- Available reference items
 - Maternal relatives
- Variation between people
 - Caucasians differ by avg 8 bases
- mtDNA is less discriminating
- Cannot interpret mixtures
- Contamination
- Heteroplasmy
- Statistics/database



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Typical Steps in mtDNA Examinations

1. Extraction
2. Amplification (PCR)
3. Post-amplification quantitation
4. Cycle sequencing
5. Sequencing (capillary electrophoresis)
6. Sequence analysis
7. Sequence comparison & interpretation
8. Database search

mtDNA Comparison & Interpretation

Specimen	Q ITEM	K ITEM 1	K ITEM 2
Range	np 15998-16389	np 15998-16389	np 15998-16389
HVI	16111 T	16111 T	16270 T
	16223 T	16223 T	16356 C
	16290 T	16290 T	
	16319 A	16319 A	
	16325 C	16325 C	
	16362 C	16362 C	
Range	np 49-408	np 49-408	np 49-408
HVII	64t	64t	73 G
	73 G	73 G	152 C
	94 A	94 A	204 C
	146 C	146 C	263 G
	153 G	153 G	315.1 C
	235 G	235 G	
	263 G	263 G	
	315.1 C	315.1 C	

Sequence Comparison

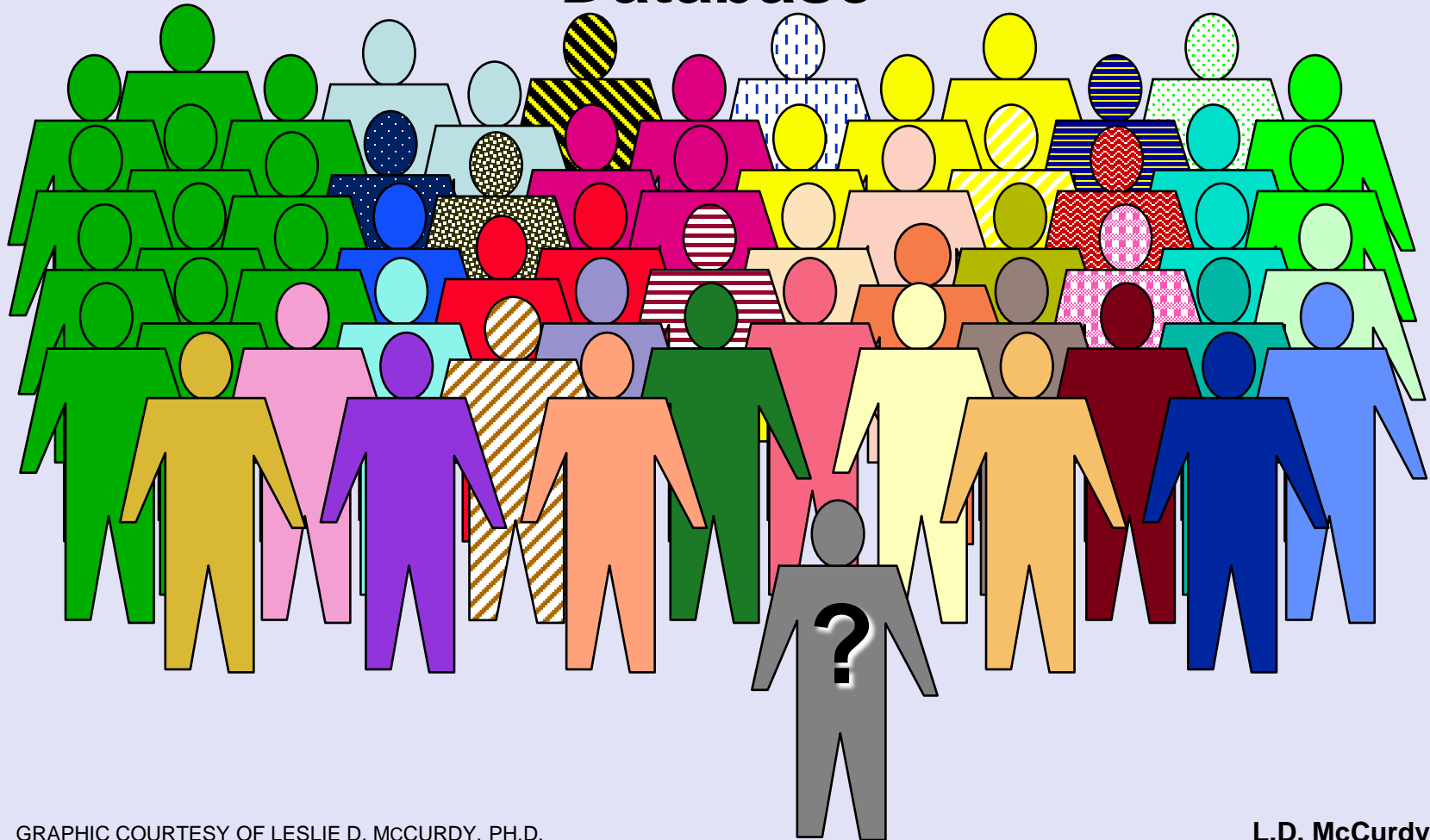
– Compare the mtDNA types between samples

Exclude

Cannot Exclude

Inconclusive

Compare Sequence to Database



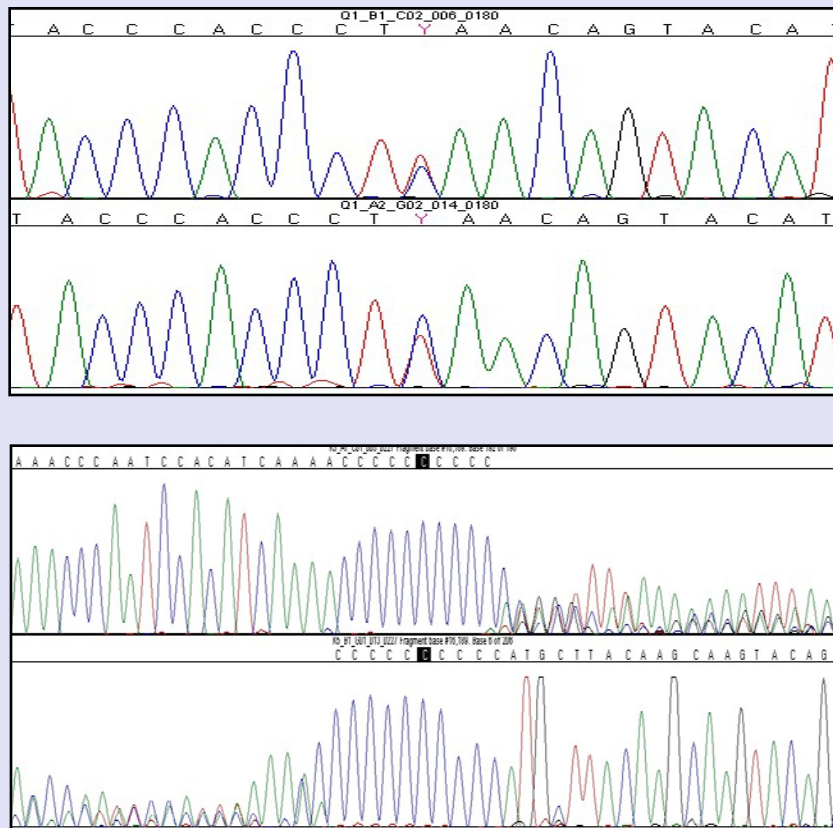
Statistics & Database

- mtDNA is a single locus
 - Bases are not independent
 - Cannot apply product rule
- Most sequences in the forensic database occur a single time (~60%)
- The true population frequencies of the majority of sequences is unknown
- Frequency estimates are limited by database size
 - Calculate upper bound frequency estimate

Heteroplasmy

The presence of more than 1 mtDNA type within an individual

- Sequence/point
 - Observation of more than 1 base at a location
- Length
 - Observation of more than single size DNA fragment
- Interpretation
 - Look for concordance
 - Single base difference?



Contamination

Minimize

- Personal protective equipment (PPE)
- Cleaning procedures
- Dedicated equipment
- Separation of space
 - Pre- and post-amplification
- Process question items before known/reference items

Monitor

- Use of controls
 - Reagent blanks, negative controls
- Establish criteria for dealing with contamination

Advantages

- Compromised samples
- Cold cases
 - Evidence types
 - Living relatives
- Available reference items
 - Direct vs indirect
- Kinship analysis
 - Missing person cases



Future

- Increased demand
- Population databases
- Increased resolution
 - Coding region variation
- Automation
- Technological advances



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