Mitochondrial DNA
• The mitochondrial genome contains ~16,569 bps
• Maternally inherited
• ~1000’s of copies per cell (tends to survive under adverse environmental conditions)
• Polymorphic control region (D-loop) (~1100 base pairs) is typically used for human identification purposes (less than 7% of total mt genome)

Mitochondrial Genome

The principal limitation in forensic mtDNA testing (using solely HV1 and HV2) is the low power of discrimination that is obtained when common “mtDNA types” are involved in a case.

Coding Region Polymorphisms
• Sequence data from mtDNA coding region reveals numerous SNPs that can help distinguish Caucasians sharing common HV types
• 10 SNP sites are being evaluated to discriminate individuals having the most common HV type (Haplogroup H-CRS)
• We are using the fluorescent primer extension assay SNaPshot for multiplex probing of coding region SNPs
• The primer extension assay allows for flexibility in designing custom multiplex assays
• Assay is run on a capillary electrophoresis platform (ABI 3100, 3100, 3700) common to most forensic laboratories

Primer Extension with SNaPshot™

Multiplex PCR Protocol
• Typical reaction volume = 15 µL
  1.2 µg of genomic DNA
  1 unit Taq Gold polymerase
  5 mM Mg²⁺
  1x Taq Gold buffer
  1 mM of each of the PCR primers
  0.16 mM dNTPs
  250 µM dNTPs
• General thermal cycling conditions (Tₙ = annealing temperature)
  Initial Tₙ = 55 °C for 3 cycles
  Increasing Tₙ = 0.2 °C for 19 cycles
  Tₙ = 55 °C for 9 cycles

Multiplexing is Achieved Through the Use of “Tailed” SNP Primers

Sequences for 10 SNP Primers

Future Goals
• SNaPshot assay development for informative sites in other common HV types
• Further testing of mtSNP 11plex (mixtures, data basing, sensitivity)
• Testing and optimizing Genotyping Macros
• Decreasing electrophoresis run time (shorter capillary, different polymers)
• Increasing the number of loci probed (12-15plex)

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