Intra-individual difference of length heteroplamy in blood and hair shaft mitochondrial DNA

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Mitochondrial DNA

- General properties
  - High copy number
  - Maternal inheritance
  - Rapid rate of evolution
- D-loop; major target for forensic field
  - Hypervariable regions (HV1, HV2 and HV3)
  - Point heteroplasmy and length heteroplasmy

Length heteroplasmy

- Occurs in HV1, HV2, and HV3 homopolymeric C-tract
- Consequence of poor replication fidelity
- More frequent than point heteroplasmy
- Limitation to profiling of length variants

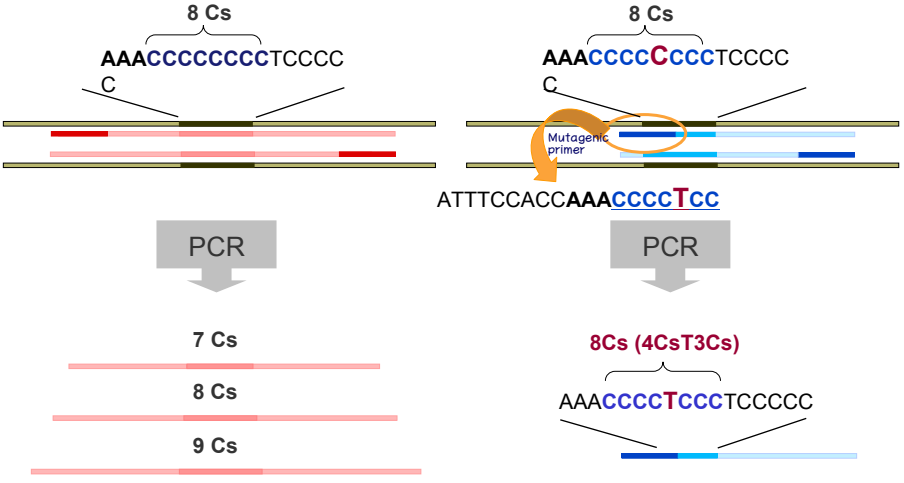
Aims of study

- Overcoming the limitation to analyze length heteroplasmy in mitochondrial HV2 region
- Analysis of intra-individual and inter-individual differences of length heteroplasmy
- Discussion on using length heteroplasmy for forensic identification

Sampling and DNA extraction

- Sampling
  - 25 individuals
  - Blood and five hair shafts
- DNA extraction
  - Using the DNA IQ™ systems (Promega) and Tissue and Hair Extraction Kit (Promega)
  - Additional decontamination step for hair shaft mtDNA extraction

Mutagenic primer design



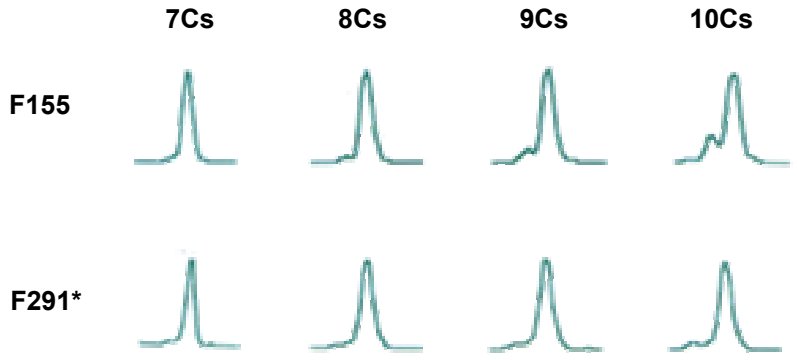
PCR amplification and detection

- PCR amplification
  - Total volume of 10.0 ul
  - Primers
    F291 (5'-ATTTCCACCAAACCCCTCC)
    R389 (5'-**HEX**-CTGGTTAGGCTGGTGTTAGG)
  - 56°C annealing temperature
  - 25 cycles for 1.0 ng of blood DNA, and 32 cycles for 0.1 ng of hair shafts mtDNA
- Size-based separation on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems)

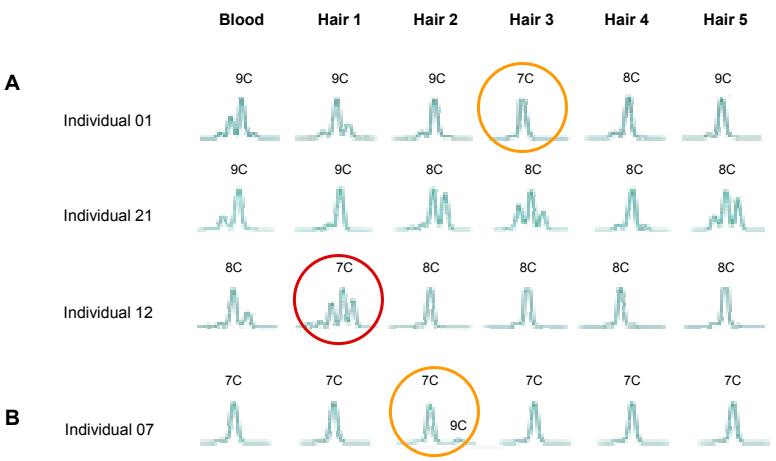
Confirmation of mitotype

- To examine the possibility of sample switching or contamination
- Subjects
  - Blood and hair shafts samples showing intra-individual difference in heteroplasmy mtDNA peak pattern
- Methods
  - Sequencing of the HV1 and HV2 region
  - Analysis of CA dinucleotide repeat length polymorphism in the HV3 region

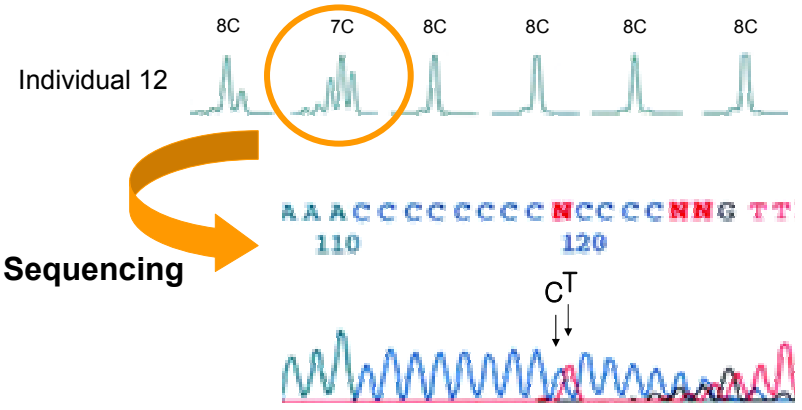
Efficiency of mutagenic primer



Electropherogram of theHV2 C-tract



Evidence of point heteroplasmy



Homoplasmic and heteroplasmic individuals

	Blood	Hair shafts		
		All homoplasmy	All heteroplamsy	Mixed*
Homoplasmy	10	7	-	3
Heteroplasmy	15	-	13	2

* Individuals who displayed homoplasmic and heteroplasmic peak patterns in hair shafts

Different major C-tract genotype

Individual	Number of cytosine in major C-tract					
	Blood	Hair 1	Hair 2	Hair 3	Hair 4	Hair 5
01	9	9	9	7	8	9
10	7	7,8	8	7	7	7
12	8	7	8	8	8	8
14	9	8	9	9	8	9
16	8	8	8	9	8	8
20	8	7	8	8	7	8
21	9	9	8	8	8	8
25	9	8	9	9	9	9

Conclusion

Analysis of length heteroplasmy in homopolymeric C-tract of the HV2 region using primer designed to minimize stutter production

- Significant variations were observed in qualitative/quantitative peak patterns in blood and hair shaft mtDNA
- Information of length heteroplasmy in a homopolymeric C-tract of the mtDNA HV2 region cannot be used alone to support an interpretation of exclusion.