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Genotyping of Nuclear Loci from Telogenic Hair Shafts Using mini-STRs

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NIST Human Identity Project Team

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Value of Hair Evidence

- Hairs are a common form of forensic evidence
- Hairs with roots are amenable to nuclear DNA testing
- Hair shafts can be examined with mtDNA (present in higher copy number)
- First demonstration of DNA from hair: Higuchi et al. (1988) DNA typing from single hairs. *Nature* 332:543-546

Root

Shaft (Telogen)

http://www.fbi.gov/hq/lab/fsc/backissu/jan2004/research2004_01_research01b.htm

Mitochondrial DNA Has More Copies Per Cell

FORENSIC SCIENCE COMMUNICATIONS
 July 1999 Volume 1 Number 2

Mitochondrial DNA Analysis at the FBI Laboratory

Figure 1 Types of DNA

Nuclear

- 2 copies/cell
- inherited from both parents
- unique to individual

Mitochondrial

- >1000 copies/cell
- maternally inherited
- not unique to individual

Nuclear DNA has a smaller number of copies per cell than mitochondrial DNA and is inherited from both parents. Mitochondrial DNA is maternally inherited without recombination and, thus, is not unique to an individual.

Disadvantages of mtDNA testing:
 Low power of discrimination
 Labor intensive
 Expensive

<http://www.fbi.gov/hq/lab/fsc/backissu/july1999/dnaf1.htm>

Nice Summary of Success Rates with mtDNA

Melton et al. (2005) *J. Forensic Sci.* 50: 73-80

J. Forensic Sci. Jan. 2005, Vol. 50, No. 1
 Paper ID JFS2004230
 Available online at: www.aafn.org

Terry Melton,¹ Ph.D.; Gloria Dimick,¹ M.S.; Bonnie Higgins,¹ M.S.; Lynn Lindstrom,^{1,2} B.S.; and Kimberlyn Nelson,¹ Ph.D.

Forensic Mitochondrial DNA Analysis of 691 Casework Hairs³⁶

Wilson, M.R., et al. (1995) Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. *Biotechniques* 18(4): 662-669.

Melton et al. (2005) obtained a full or partial mtDNA profile for >92% of hairs tested

PCR Product Size Reduction Improves Recovery of STR Information from Telogen Hairs

Hellmann, et al. (2001) *Int. J. Legal Med.* 114(4-5): 289-273

Int J Legal Med (2001) 114:269-273 © Springer-Verlag 2001

TECHNICAL NOTE

First use of miniSTRs for typing hair shafts

A. Hellmann · U. Rohleder · H. Schmitter · M. Wittig

STR typing of human telogen hairs – a new approach

108 bp size reduction

160 bp size reduction

Fig. 2 Typing efficiency (%) of single telogen hairs using v-primer pairs (TH01), n = 49 in comparison to primer pairs supplied by the AmpfSTR Green I PCR Amplification kit (TH01)gk, n = 48 (36 from preliminary experiments) for the HUMTH01 locus

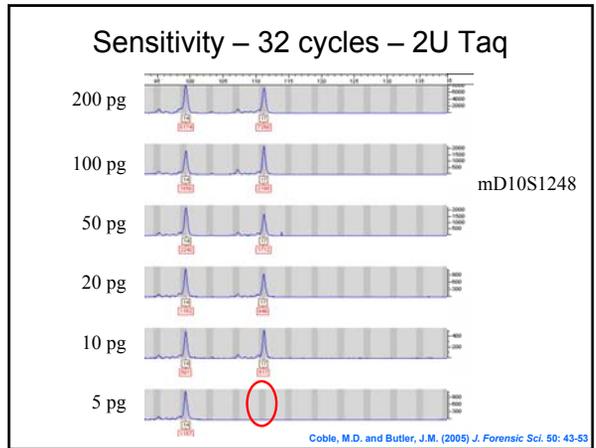
Fig. 4 Typing efficiency (%) of single telogen hairs using v-primer pairs (TPOX), n = 49 in comparison to primer pairs supplied by the AmpfSTR Green I PCR Amplification kit (TPOX)gk, n = 48 (36 from preliminary experiments) for the HUMTPOX locus

New Higher Sensitivity Nuclear DNA Assays (miniSTRs)

Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50: 43-53 *J. Forensic Sci.* Jan. 2005, Vol. 50, No. 1
Paper ID JFS2004216
Available online at: www.astrn.org

Michael D. Coble,¹ Ph.D. and John M. Butler,¹ Ph.D.

Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA*



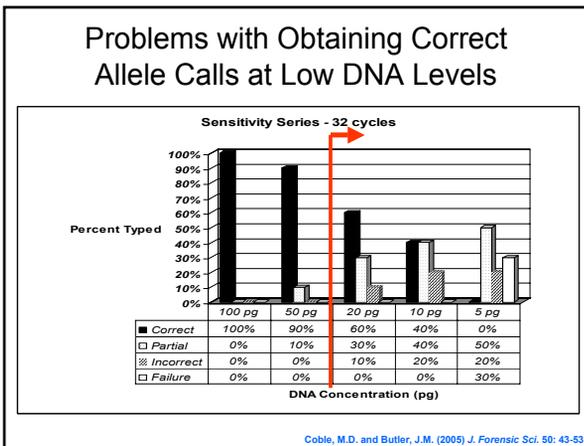
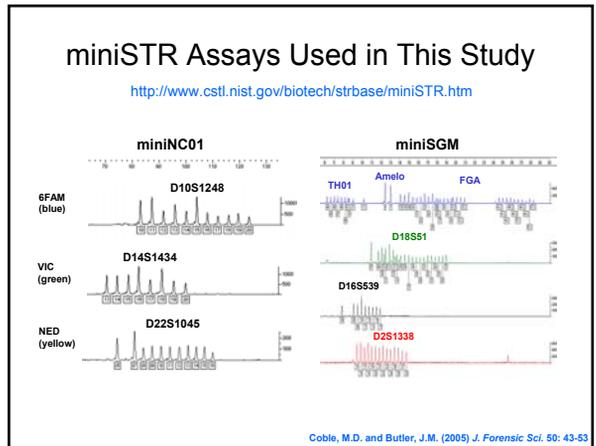
Interpretational Issues with LCN

2 Amplifications – report only the alleles observed in both amps.

→

~ 30 µL

2 miniSTR tests
Quantifier
SNP testing
mtDNA amplification



Extraction Methods

"Standard Protocol"
Micro Tissue Grinding in Extraction Buffer

Complete Hair Shaft Digestion

Int J Legal Med (2001) 114:269–273 © Springer-Verlag 2001

TECHNICAL NOTE

A. Hellmann · U. Rohleder · H. Schmitter · M. Wittig
STR typing of human telogen hairs – a new approach

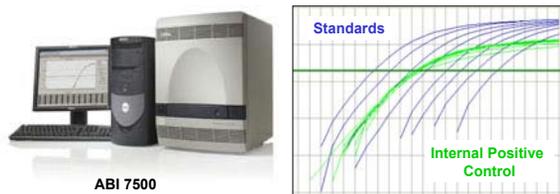
Can be done is as little as 1 hour but usually run overnight

TN_{Ca} Buffer
 Tris 2%SDS
 NaCl ProK
 CaCl₂ DTT

Methods Attempted for Extraction of DNA from Hair Shafts

- Organic Extraction (Phenol Chloroform)
- Qiagen
- ChargeSwitch – Magnetic bead assay to capture DNA.

DNA Quantitation with Real-Time PCR



ABI 7500

Quantifier assay

Can Now Examine Low Levels of Nuclear DNA...

Obtaining Hair Samples

- AFDIL – Kim Murga, Demris Lee, and Col. Brion Smith.
- NIST – Call for Volunteers

Call for Volunteers

This Researcher couldn't find volunteers for his hair study...



Collection Kit – Bode Buccal Swab (reference), Paper envelope for head hair.
 Age and Chemical treatment status of hair was requested of the volunteer.

21 Individuals - age Range – 16-71
 Analyzed 5 hairs from each person (106 total) including quant. data

Protocol

- Hairs were measured and examined under the microscope for root (removed + 1.5 cm).
- Hairs were cleaned using a 10% Triton X100 wash with sonication for 20 minutes (2X).
- 400 µL of TN_{Ca} buffer was added and tubes were incubated overnight at 56°C.
- Extraction with Phenol Chloroform and concentration with Microcon YM30 (Millipore).

Long Term Effects of Phenol??



	MicroTissue Grinding		
	D10S	D14S	D22S
PV 2.5cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 2.5cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 2.7cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 2.7cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 5 hairs	10ul	-	-
	5ul	-	-
	2.5ul	-	08, 08
PV 5 hairs	10ul	-	-
	5ul	-	-
	2.5ul	-	-
KEC 20cm	10ul	-	-
	5ul	-	-
	2.5ul	-	13, 13
MK 18cm	10ul	-	-
	5ul	-	-
	2.5ul	14, 15 (<50)	17, 18, 08, 12

MicroTissue Grinding Protocol

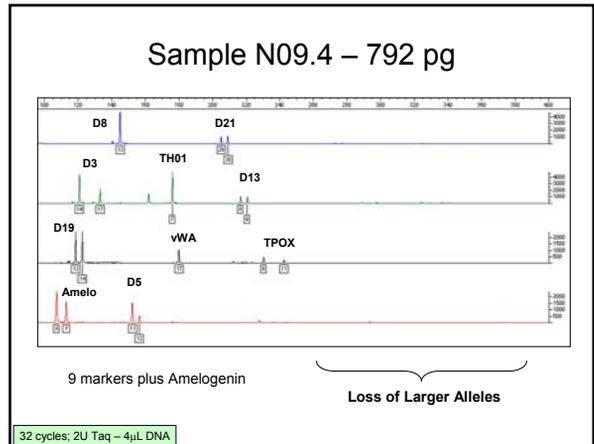
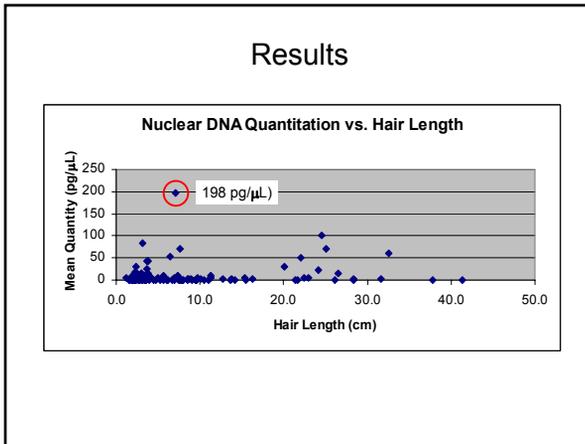
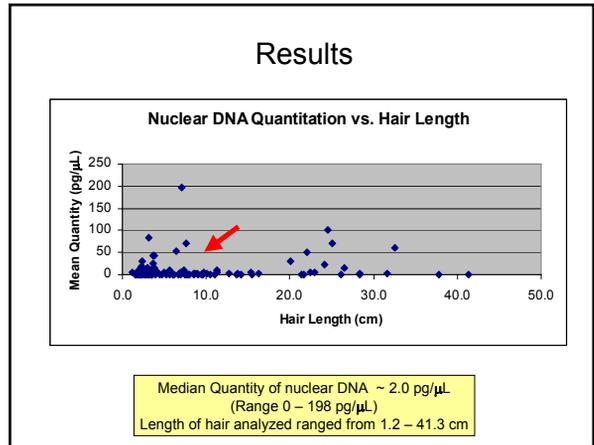
32 cycles; 2U Taq

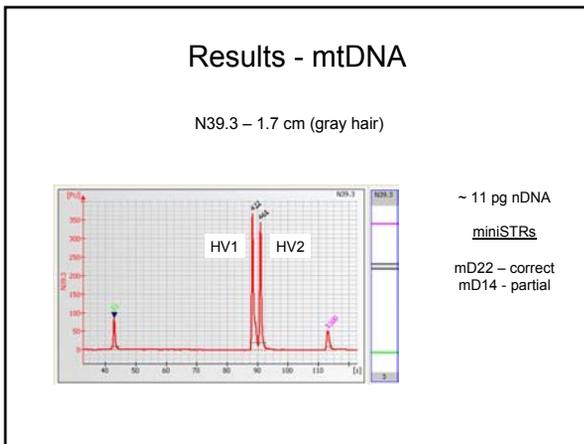
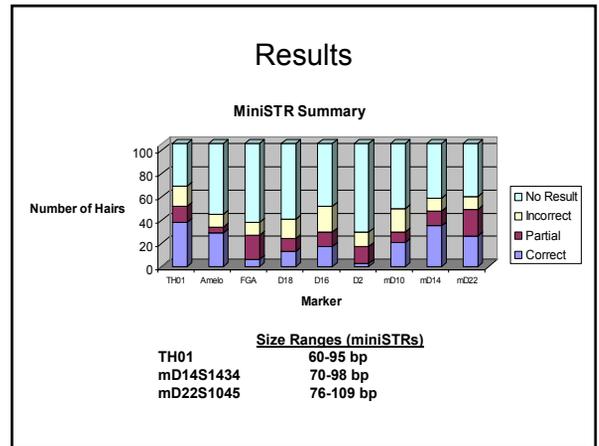
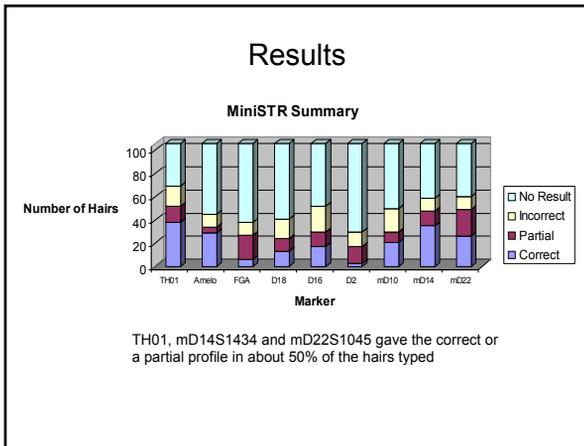
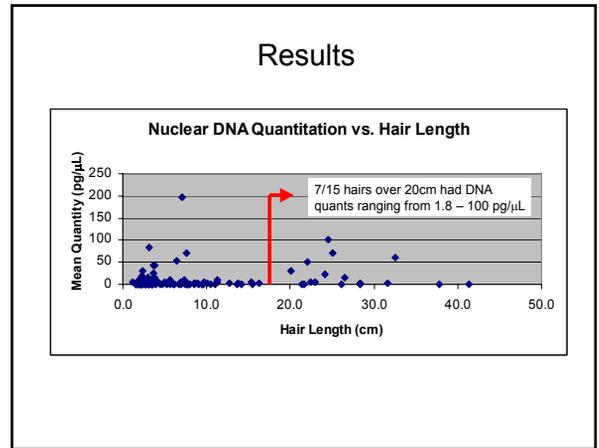
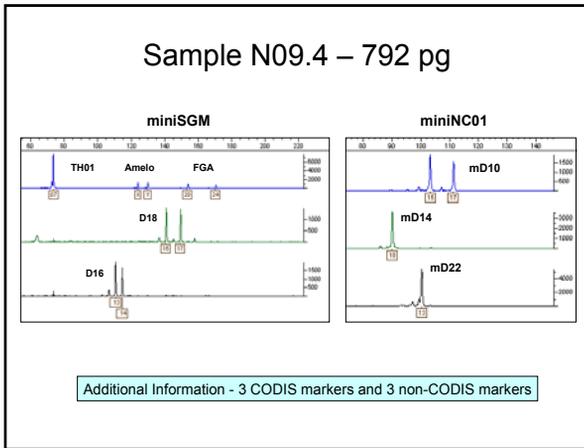
	Complete Digestion		
	D10S	D14S	D22S
PV 2.5cm	10ul	-	08, 13
	5ul	-	08, 08
	2.5ul	-	-
PV 2.5cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 2.7cm	10ul	-	-
	5ul	-	-
	2.5ul	-	-
PV 2.7cm	10ul	-	08, 13 (<50)
	5ul	-	13, 13
	2.5ul	-	-
PV 5 hairs	10ul	16, 16	17, 18, 08, 13
	5ul	16, 16	17, 18, 08, 08
	2.5ul	15, 15	17, 18, 08, 13
PV 5 hairs	10ul	-	08, 13
	5ul	-	08, 13
	2.5ul	-	13, 13
KEC 20cm	10ul	16, 17	17, 13
	5ul	16, 17	17, 13
	2.5ul	16, 17	17, 13
MK 18cm	10ul	14, 15	17, 18, 08, 12
	5ul	14, 15	17, 18, 08, 12
	2.5ul	14, 15	17, 18, 08, 12
Genotypes	D10S	D14S	D22S
PV	15, 16	17, 18	08, 13
KEC	16, 17	17, 17	13, 13
MK	14, 15	17, 18	08, 12

Complete Digestion Protocol

32 cycles; 2U Taq

"Longer" Hairs – greater success





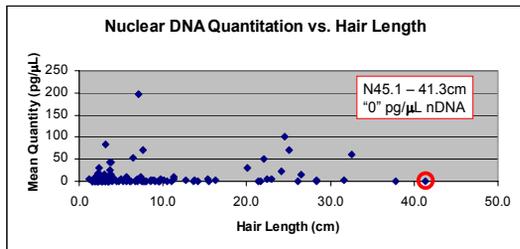
Inhibition of PCR - Melanin

Biochem. and Biophys. Res. Comm. **271**, 726-730 (2000).

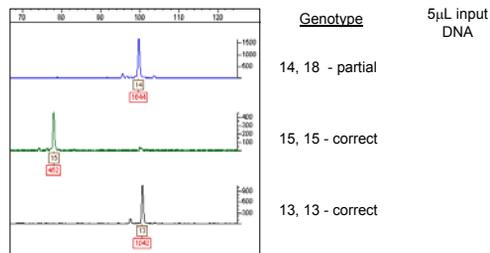
Melanin Binds Reversibly to Thermostable DNA Polymerase and Inhibits Its Activity

Leopold Eckhart* Jürgen Bach* Jozef Ban*† and Erwin Tschachler* ‡§

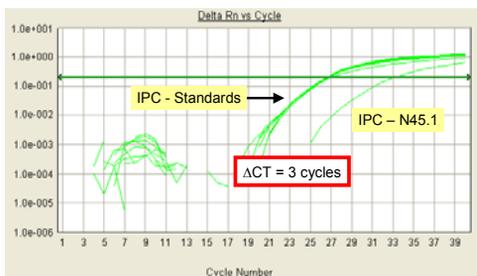
Inhibition of PCR - Melanin



Inhibition of PCR - Melanin



Inhibition of PCR - Melanin



Conclusions

- Small amounts of nuclear DNA are present in hair shafts.
- The amount recovered varies widely among hairs/individuals.
- Markers having small size ranges (<100 bp) gave better results.
- When above 50 pg total nDNA, miniSTR or SNP profiles can be obtained.

Future Studies

- Melanin Removal – Ken Rubinson (NIST).
- Identification of additional miniSTR markers with ranges <100 bp.

Funding and Collaborations

We are funded by an Interagency Agreement between **National Institute of Justice** and NIST Office of Law Enforcement Standards

Our publications and presentations are made available at:
<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>

Human Identity Project Team

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 Margaret Kline
 Jan Redman
 Amy Decker
 David Duewer

Collaborators (also funded by NIJ):

Bruce McCord and students (Ohio U) for miniSTR work

Kim Murga, Demris Lee, Col. Brion Smith and everyone at **AFDIL** for hair collection and extraction protocols.

Wille May (CSTL Director)

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