Novel Methods to Obtain Nuclear DNA Profiles from Rootless Hair Shafts

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Introduction

Hair samples are a common form of trace evidence in many forensic laboratories, and can oftentimes link an individual to a crime or crime scene. Forensic laboratories typically first analyze hair evidence for the presence of root material. If present, nuclear DNA (nuDNA) profiling may be attempted with conventional methods, such as STR typing. However, if root material is lacking, forensic laboratories often do not process the sample, or submit it for mitochondrial DNA (mtDNA) sequencing. While typing of nuDNA from hair shafts has often been unsuccessful previously, the presence of some nucleated biologically dead cells or keratinocytes in their last stage of differentiation on a hair shaft make it possible to extract nuDNA[1]. This DNA is both degraded and low in copy number, however, making it difficult to obtain informative profiles with conventional nuDNA testing methods.

We report here utilization of a novel extraction methodology[4] for hairs in combination with a recently developed typing technology to improve analysis success rate from highly compromised samples. The InnoTyper® 21 kit is a small amplicon (~60-125bp) *Alu* based multiplex[2] that is compatible with currently used PCR/CE platforms, which can be utilized for DNA typing of highly degraded and/or low-level samples, such as hair shafts.

Materials and Methods

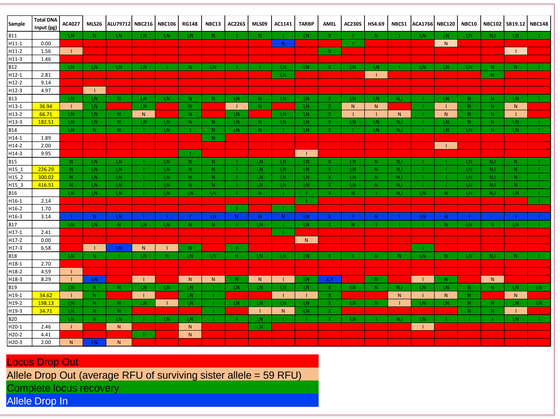
- Hair sample collection and preparation: samples were collected from a total of 20 individuals. Each donor provided three hairs and a reference buccal swab. Each hair was viewed under a stereomicroscope to identify the presence of a follicular tag. The tag (0.5cm) was removed with UV irradiated scissors and discarded. A 2 cm fragment of hair was then cut from this end. Hairs were cleaned via sonication in 5% Terg-a-zyme for 20 minutes, followed by a brief rinse in 100% ethanol, then molecular biology grade water.
- Modified DNA extraction: Cleaned hairs were digested in Qiagen® Buffer ATL with Proteinase K and DTT for 1 hour. Qiagen® Buffer AL was then added and samples incubated for a further 10 minutes. Samples were then purified using the Applied Biosystems® PrepFiler™ BTA Forensic DNA Extraction Kit.
- DNA quantification: DNA extracts were quantitated using degradation assessment qPCR quantitation kits, InnoQuant®[3] and/or Quantifiler® Trio, allowing determination of the degradation index (DI = [short]/[long])
- PCR Amplification and typing: Samples were amplified using the novel small amplicon system InnoTyper® 21. A subset of the hair samples were also amplified with the GlobalFiler® PCR Amplification Kit. AB 3130 Genetic Analyzer was utilized. Data analysis was performed with GMID-X.

Results

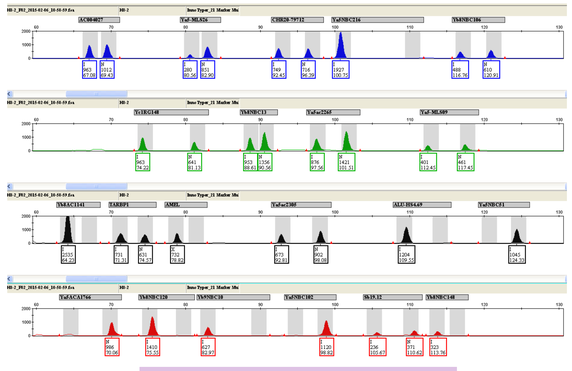
Allele Recovery with InnoTyper® 21

Sample ID	DI	Input DNA (pg)	IT21 % Allele Recovery	Sample ID	DI	Input DNA (pg)	IT21 % Allele Recovery
Batch 1:							
1-1	-	0.7	12%	6-1	-	1.3	10%
1-2	-	0.7	10%	6-2	-	1	14%
1-3	-	1	5%	6-3	-	1.3	N/A
2-1	7	102.5	74%	7-1	-	0.6	14%
2-2	66	56.3	55%	7-2	-	1.6	12%
2-3	28	112.1	100%	7-3	-	1.6	2%
3-1	-	1.5	5%	8-1	19	77.2	90%
3-2	-	2.6	0%	8-2	5	67.2	100%
3-3	-	1.1	7%	8-3	17	94	98%
4-1	16	251.3	100%	9-1	52	106.8	98%
4-2	22	176.6	98%	9-2	-	73.8	88%
4-3	17	102.4	98%	9-3	-	46.1	60%
5-1	-	2.6	19%	10-1	105	101.5	76%
5-2	-	5.7	24%	10-2	63	129.1	93%
5-3	-	3.9	14%	10-3	114	139.9	76%
Batch 2:							
11-1	-	0	10%	16-1	-	2.14	10%
11-2	-	1.56	10%	16-2	-	1.7	10%
11-3	-	1.46	0%	16-3	-	3.14	N/A
12-1	-	2.81	12%	17-1	-	2.41	5%
12-2	-	9.14	0%	17-2	-	0	2%
12-3	-	4.97	2%	17-3	-	6.58	26%
13-1	-	36.94	55%	18-1	-	2.7	0%
13-2	-	66.71	67%	18-2	-	4.59	2%
13-3	29	182.51	100%	18-3	-	8.29	48%
14-1	-	1.89	5%	19-1	-	34.62	43%
14-2	-	2.16	2%	19-2	-	198.13	95%
14-3	-	9.95	7%	19-3	-	34.71	45%
15-1	17	226.29	100%	20-1	-	2.46	14%
15-2	29	300.02	100%	20-2	-	4.41	7%
15-3	16	416.51	100%	20-3	2	2.1	10%
Over 40% Genotype Recovery				Less than 40% Genotype Recovery			

42% of all hairs tested produced allele recoveries greater than 40% with InnoTyper® 21



InnoTyper® 21 Results from 2 cm hair shaft:



Total DNA = 67 picograms
100% Allele Recovery
Degradation Index = 5

InnoTyper® 21 and GlobalFiler® Comparison (Batch 2 only)

	IT21	GF
Samples with > 40% allele recovery	33%	0%
Samples with no allele activity detected	7%	77%
Samples with some allelic activity	87%	23%
Samples with allelic activity that were full profiles	15%	0%
Samples with allelic activity exhibiting allele or locus dropout	85% Avg RFU of sister allele = 59 RFU	100%
Samples exhibiting allele drop-in	13% Avg RFU of drop-in alleles = 74 RFU	10%
Samples exhibiting contamination	3%	0%

InnoTyper® yields higher allele recovery due to small amplicon size

InnoTyper® 21 provides higher statistical power due to higher allele recovery

Hairs H15 full IT21 profile = ***1 in 132 billion Caucasians*** *from a 2 cm rootless hair shaft!*

Sample	Total DNA Input (pg)	IT21 Match Prob AA	IT21 Match Prob Cauc	GF Match Prob AA	GF Match Prob Cauc
H13-1	36.94	3 thousand	603 thousand	0	0
H13-2	66.71	299 thousand	1 million	2	3
H13-3	182.51	94 million	68 billion	1.2 thousand	6.6 thousand
H15_1	226.29	5 billion	132 billion	25 million	6 million
H15_2	300.02	5 billion	132 billion	436 thousand	20 thousand
H15_3	416.51	5 billion	132 billion	3 billion	177 million
H19-1	34.62	1.2 thousand	208	0	0
H19-2	198.13	38 million	179 million	6.6 thousand	16 thousand
H19-3	34.71	12 thousand	19 thousand	0	0

Conclusions

- Highly discriminatory nuDNA results can be obtained from rootless hair shafts.
- In spite of hair shafts exhibiting both LCN and degradation, over 40% of the samples produced interpretable profiles.
- Obtaining a profile was largely dependent on DNA quantity AND Degradation Index – InnoQuant® was very predictive
- Hair shafts processed with InnoTyper® 21 provided usable genotype data with stronger statistical power than mtDNA
- InnoTyper® is highly sensitive and has a high discrimination power (in the billions), thus eliminating the need to resort to mtDNA sequencing for hair shaft samples.
- InnoTyper® 21 provides a way for forensic labs to efficiently process hair shafts using existing PCR/CE platforms

Literature cited

1. Lynch CA. Degeneration of Nuclei and Mitochondria in human hairs, Journal of Forensic Science 2009;54(2):346-349.
2. S.K. Sinha et al. *Development of a Novel and Sensitive DNA Analysis Multiplex Based on INNUL Markers for Highly Degraded Forensic DNA Samples*. Oral presentation at the 66th Annual Meeting of the AAFS. Seattle, WA.
3. G.M. Pineda et al. *Development and validation of InnoQuant™, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements Alu and SVA*. Forensic Science International: Genetics 13 (2014) 224–235.
4. E.S. Burnside, B.J Bintz, M.R Wilson. Improved extraction efficiency of human mitochondrial DNA from hair shafts and its implications for sequencing of the entire mtGenome from a single hair fragment, in: Proceedings of the American Academy of Forensic Sciences 65th Annual Meeting, Washington, DC, February 18-23 2012

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant No. 1230352. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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