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Alternative DNA Profiling Strategies for Rootless Hair Shafts

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INTRODUCTION

Though commonly found at crimes scenes hairs are often underutilized as evidence. In particular rootless hair shafts are a challenging sample type that contain both low template and degraded DNA. [1] During the telogen phase hairs are naturally shed with a clubbed root containing little to no additional genetic material in comparison to the hair shaft. Once recovered, these samples may lead to unsuccessful genotyping results using traditional short tandem repeat (STR) analysis with capillary electrophoresis (CE). [2] However, there are alternative forensic testing workflows that can give probative DNA results.

Success with rootless hair shafts depends on the recovery of highly degraded DNA. Amplification of this recovered DNA using PCR chemistries based on small amplicon targets further increases the chances for successful sample processing.[2,3] In this research a novel hair extraction chemistry from InnoGenomics Technologies was used in conjunction with a variety of methods for DNA analysis. Included as analysis options were traditional STR typing, CE based mitochondrial sequencing, CE based bi-allelic assays, and massively parallel sequencing (MPS) based nuclear and mitochondrial typing.

MATERIALS AND METHODS

Sample Collection

- Reference buccal swabs
- Hair from individual hair brushes

Sample Preparation

- 0.5 cm from proximal end removed
- 2 cm of hair shaft measured for extraction

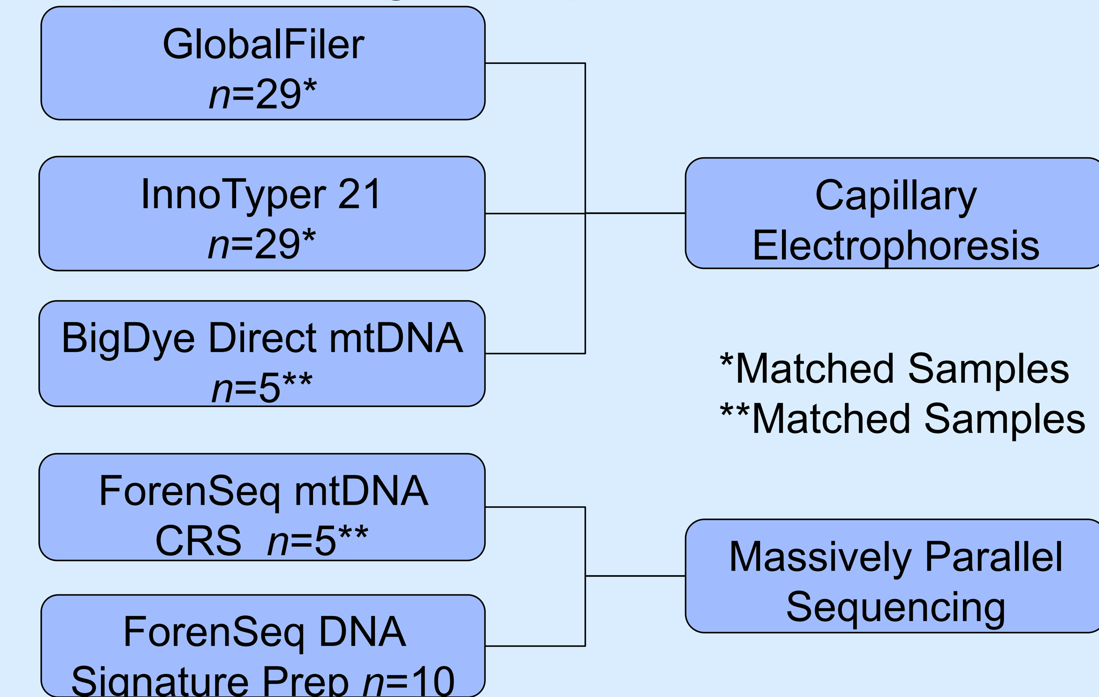
Sample Extraction

- Washed (*n*=10) and Unwashed (*n*=34) rootless hair was processed with the InnoGenomics Hair Extraction Kit (New Orleans, Louisiana)
- Eluted in 40 µL of TE buffer

Sample Quantification

- Samples were quantified with InnoQuant HY on an Applied Biosystems 7500 Real-Time PCR System

Sample Processing Techniques



RESULTS AND DISCUSSION

Mitochondrial Sequencing

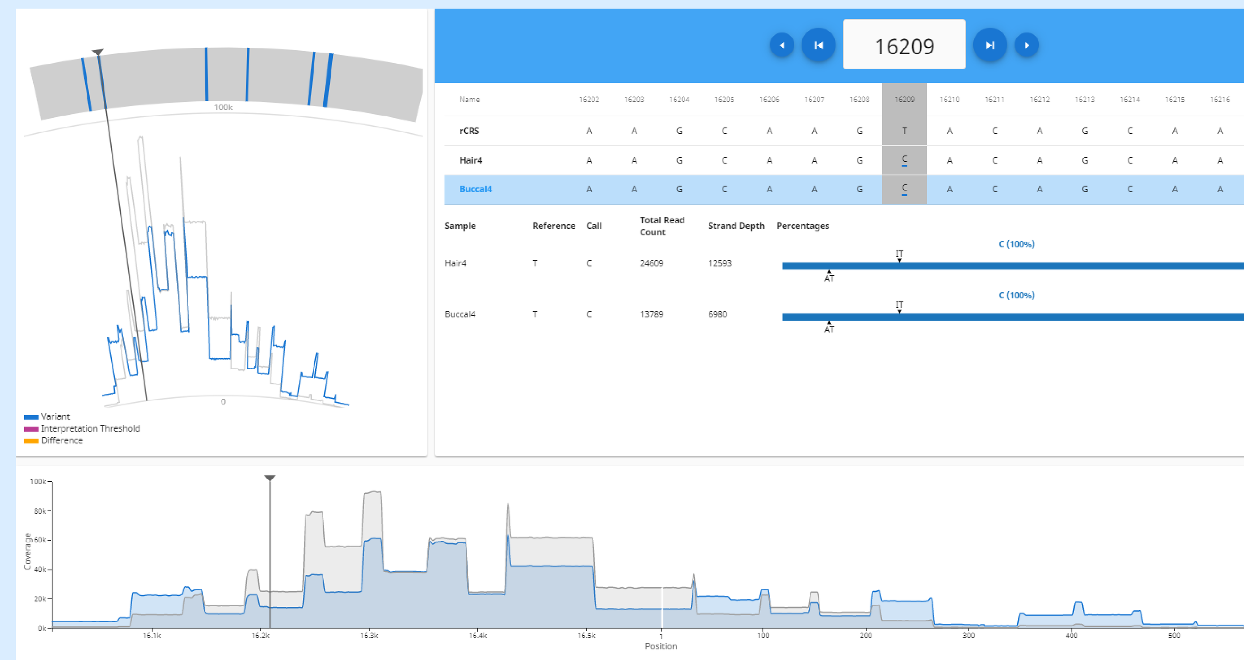


Figure 1: Analysis of 2 cm unwashed rootless hair using the ForenSeq mtDNA Control Region Solution on an Illumina MiSeq FGx. Hair shaft extracts (*n*=5) were concordant to profiles obtained using Sanger Sequencing as well as reference profiles generated using buccal swabs.

CE Recover by Amplicon Length

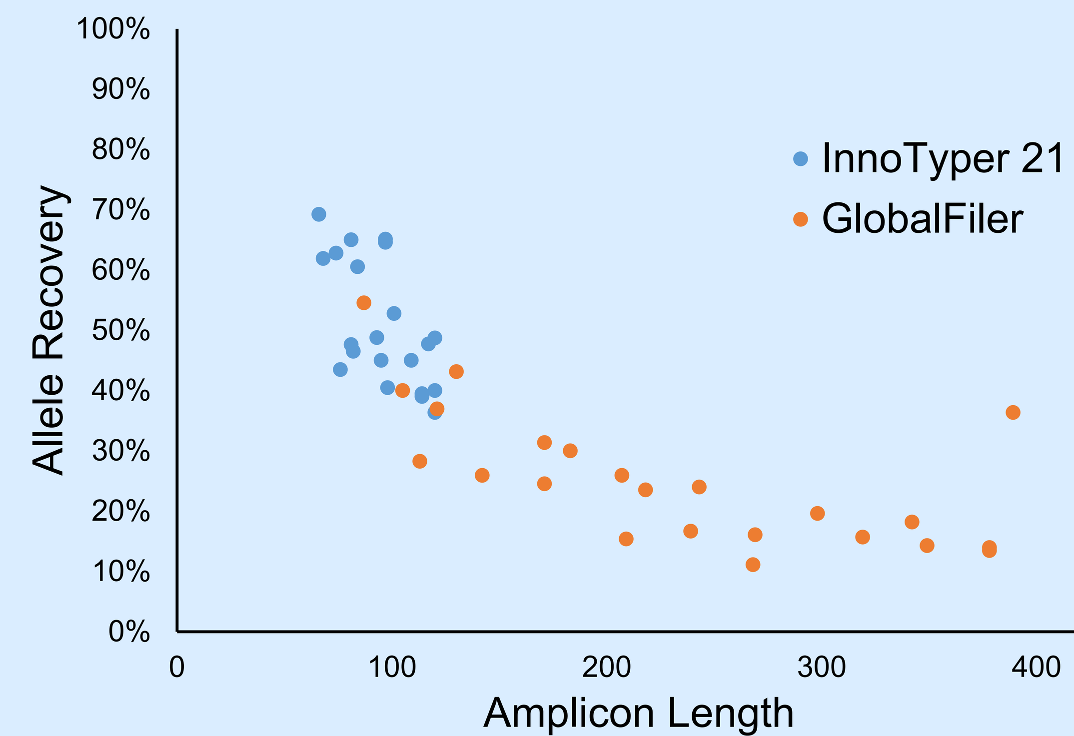


Figure 2: Alleles recovered as a function of amplicon length using Applied Biosystem's 3500 Genetic Analyzer (*n*=29). Amplicon size is based on largest possible allele.

Ancestry Estimation

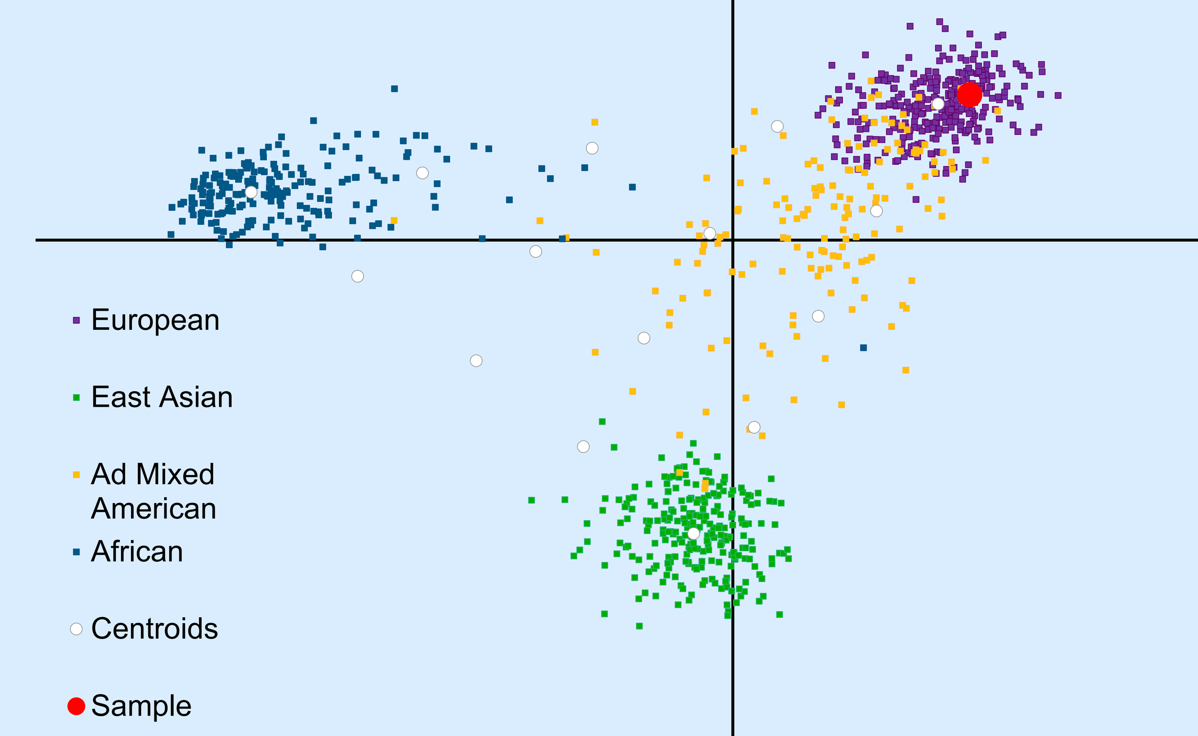


Figure 3: Primary Component Analysis plot generated using the ForenSeq Universal Analysis Software to estimate ancestry.

1000 Genomes Populations with Samples in Centroid with Sample

Population	Hair	Reference	Training Data
British in England and Scotland	68	69	70
Finnish in Finland	64	71	75
Puerto Ricans from Puerto Rico	11	7	52
Colombians from Medellin, Colombia	13	8	50
Iberian population in Spain	6	6	6
Utah Residents (CEPH) with Northern and Western European ancestry	69	70	72
Mexican Ancestry from Los Angeles USA	3	2	54
Toscani in Italia	79	87	98

Table 1: Ancestry SNPs used during ancestry estimation. Data was generated using ForenSeq DNA Signature Prep Kit primer set B on an Illumina MiSeq FGx. Estimation was successful for 2 of 10 test samples.

Profile Recovery

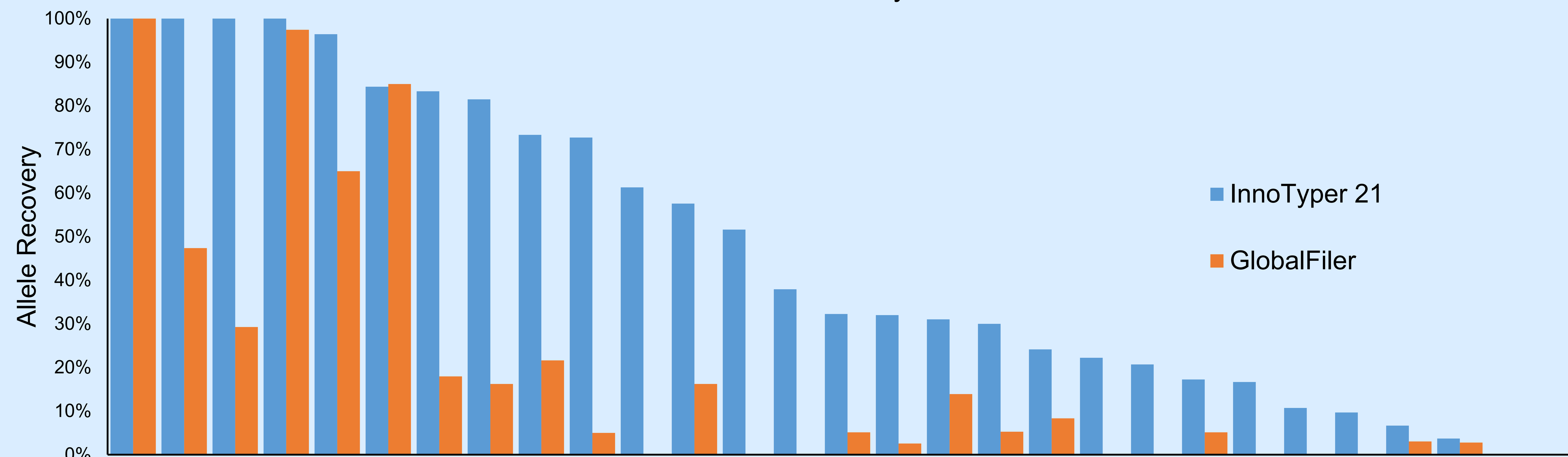


Figure 4: Comparison of profile completeness between InnoTyper 21 and GlobalFiler using an Applied Biosystem's 3500 Genetic Analyzer. Analyzed with Genemapper ID-X v1.4 (*n*=29). InnoTyper Profiles averaged 50.2% completeness while GlobalFiler averaged 22% completeness.

CONCLUSIONS

- The Innogenomics Hair Extraction Chemistry provides extracts suitable for use with multiple amplification chemistries and common instrumentation
- InnoTyper 21 amplicons amplify a wider range of hair sample extracts when compared to GlobalFiler
- Allele drop in (GlobalFiler=2, InnoTyper=12) requires an intentional methodology to balance between sample pre-processing and profile recovery
- Amplification chemistries designed for the MiSeq FGx contain amplicon sizes suitable for rootless hair shaft analysis
- The ForenSeq mtDNA Control Region Solution is concordant with current CE based Sanger Sequencing; for more visit poster number 68

REFERENCES

[1] D. McNevin, L. Wilson-Wilde, J. Robertson, J. Kyd, C. Lennard, Short tandem repeat (STR) genotyping of keratinised hair: Part 1. Review of current status and knowledge gaps, Forensic Science International 153(2) (2005) 237-246.

[2] K.S. Robertson, D. McNevin, J. Robertson, STR genotyping of exogenous hair shaft DNA, Australian Academy of Forensic Sciences, Australia, 2007, p. 107.

[3] K.S. Grisedale, G.M. Murphy, H. Brown, M.R. Wilson, S.K. Sinha, Successful nuclear DNA profiling of rootless hair shafts: a novel approach, International Journal of Legal Medicine 132(1) (2018) 107-115.

ACKNOWLEDGEMENTS

This research was conducted in accordance with the guidance of the Sam Houston State University Institutional Review Board for the Protection of Human Subjects. This project was supported by the Forensic Sciences Foundation through the Lucas Grant Program. In addition the authors would like to acknowledge the support of the Department of Forensic Science at Sam Houston State University.