

DNA Profiling of Rootless Hair Shafts Utilizing Massively Parallel Sequencing and Bi-Allelic Assays

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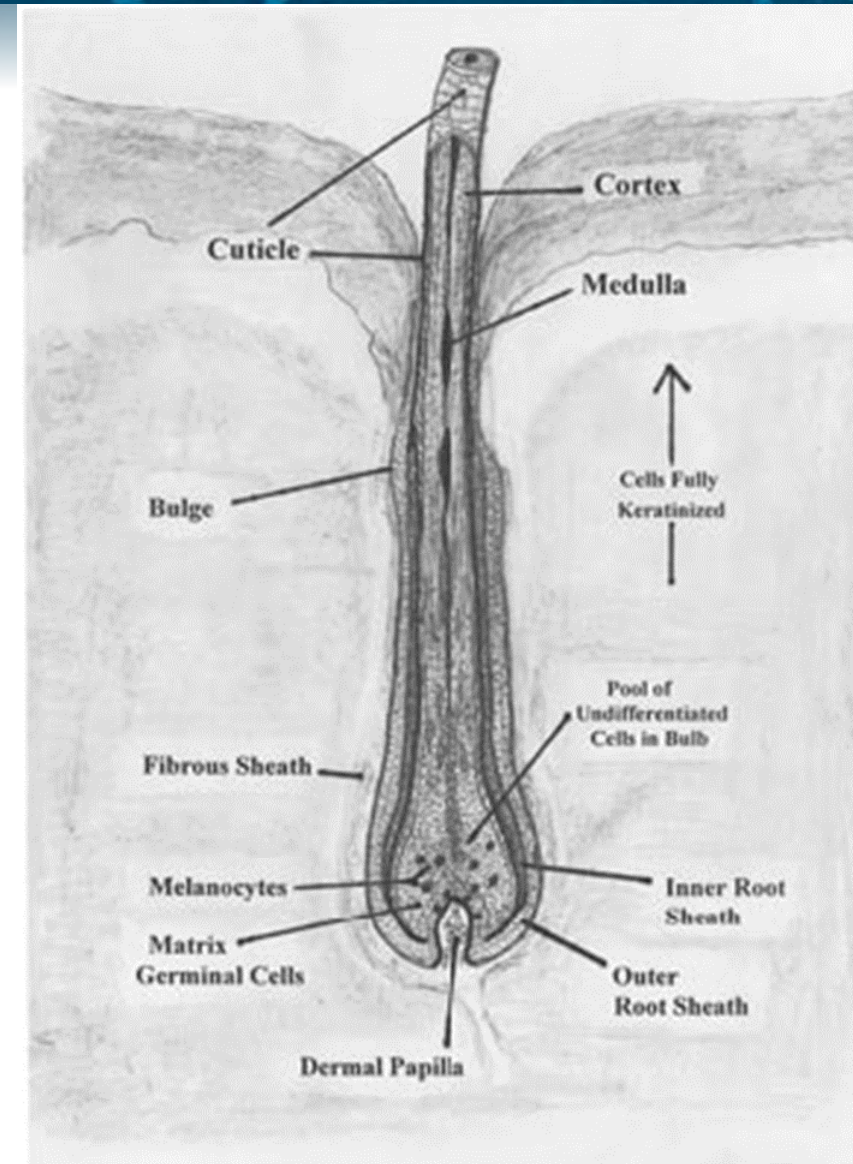


FSF Emerging Forensic Scientist Award
Paper Presentation

Limitations of Genetic Testing

Challenges commonly seen in biological samples

- Low template samples
- Degraded samples
- Inhibited samples
- Mixture samples



Introduction Cont.

Advantages of Hair

- Persistence
- Can be decontaminated
- Commonly collected as evidence

STR typing of ancient DNA extracted from hair shafts of Siberian mummies

S. Amory^{a,b,*}, C. Keyser^{a,b}, E. Crubézy^b, B. Ludes^a

Paleo-Eskimo mtDNA Genome Reveals Matrilineal Discontinuity in Greenland

M. Thomas P. Gilbert,¹ Toomas Kivisild,² Bjarne Grønnow,³ Pernille K. Andersen,⁴ Ene Metspalu,⁵ Maere Reidla,⁵ Erika Tamm,⁵ Erik Axelsson,¹ Anders Götherström,⁶ Paula F. Campos,¹ Morten Rasmussen,¹ Mait Metspalu,⁵ Thomas F. G. Higham,⁷ Jean-Luc Schwenninger,⁷ Roger Nathan,⁷ Cees-Jan De Hoog,⁸ Anders Koch,⁹ Lone Nukaaraq Møller,^{10*} Claus Andreasen,¹¹ Morten Meldgaard,¹² Richard Villems,⁵ Christian Bendixen,⁴ Eske Willerslev^{1†}

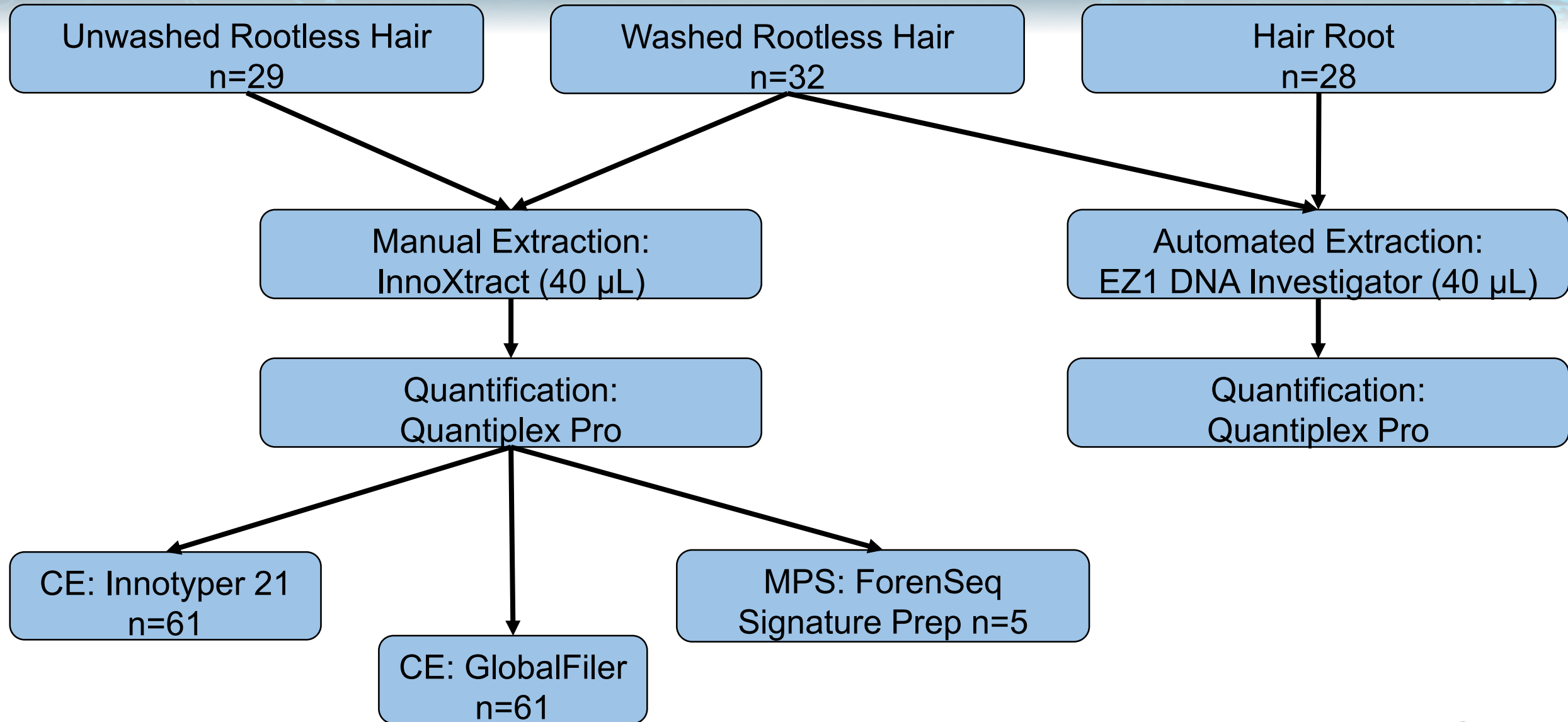
Resistance of degraded hair shafts to contaminant DNA

M Thomas P Gilbert^{a,*}, Laura Menez^{a,b}, Robert C Janaway^c,
Desmond J Tobin^d, Alan Cooper^a, Andrew S Wilson^{c,d}

Goal

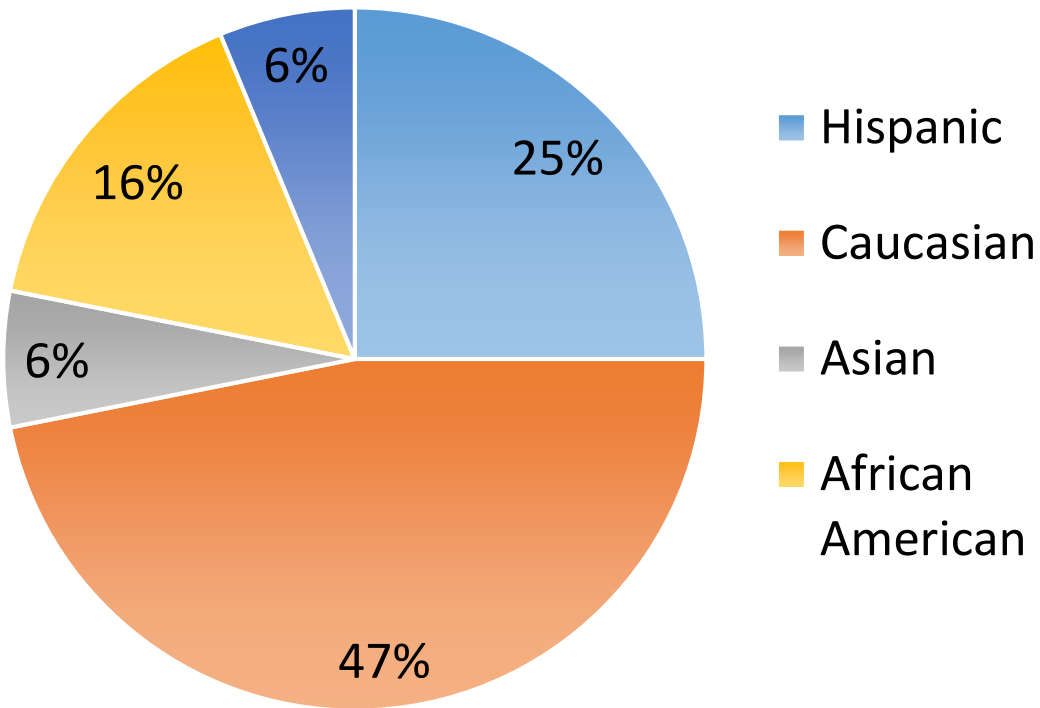
- Bolster DNA recovery from rootless hair samples (specifically, a single 2 cm shaft)
 - Extraction method
 - Sample preparation
- Test amplification alternatives to traditional CE genotyping while retaining enough extract for mtDNA genotyping
 - InnoTyper 21- Capillary Electrophoresis
 - ForenSeq DNA Signature Prep- Massively Parallel Sequencing

Experimental Design

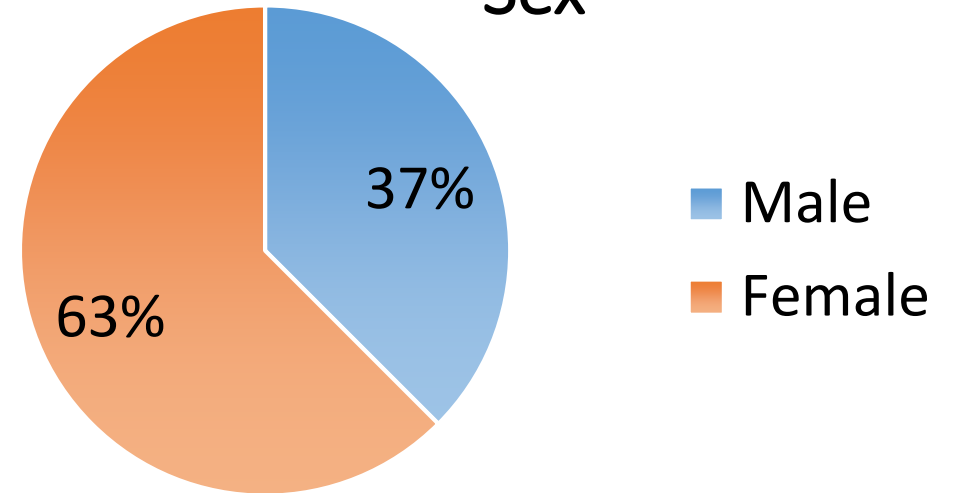


Sample Population n=32

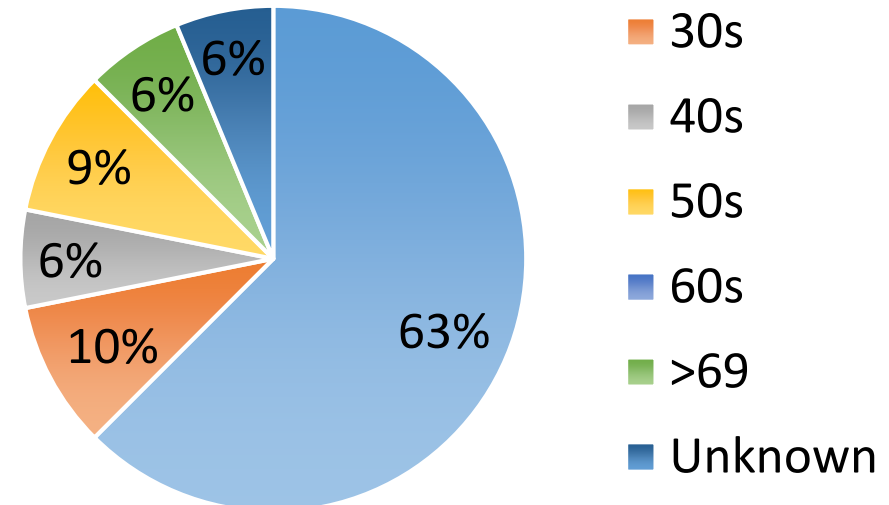
Ancestry



Sex



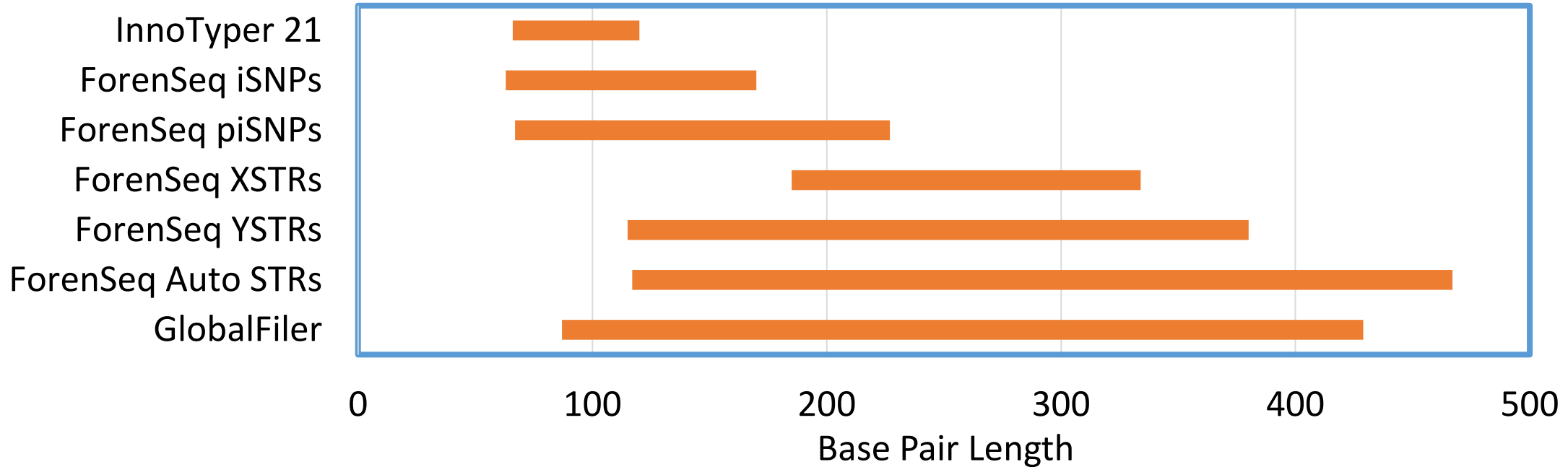
Age



- Hairs collected from personal hair brushes
- Buccal swabs for reference profiles
- Cleaned following adapted Grisedale et. al method

Comparison of Technologies

Amplicon Size



GlobalFiler

- 21 Autosomal STRs
 - DYS319
 - Amelogenin
 - Y-Indel
- 24 Total

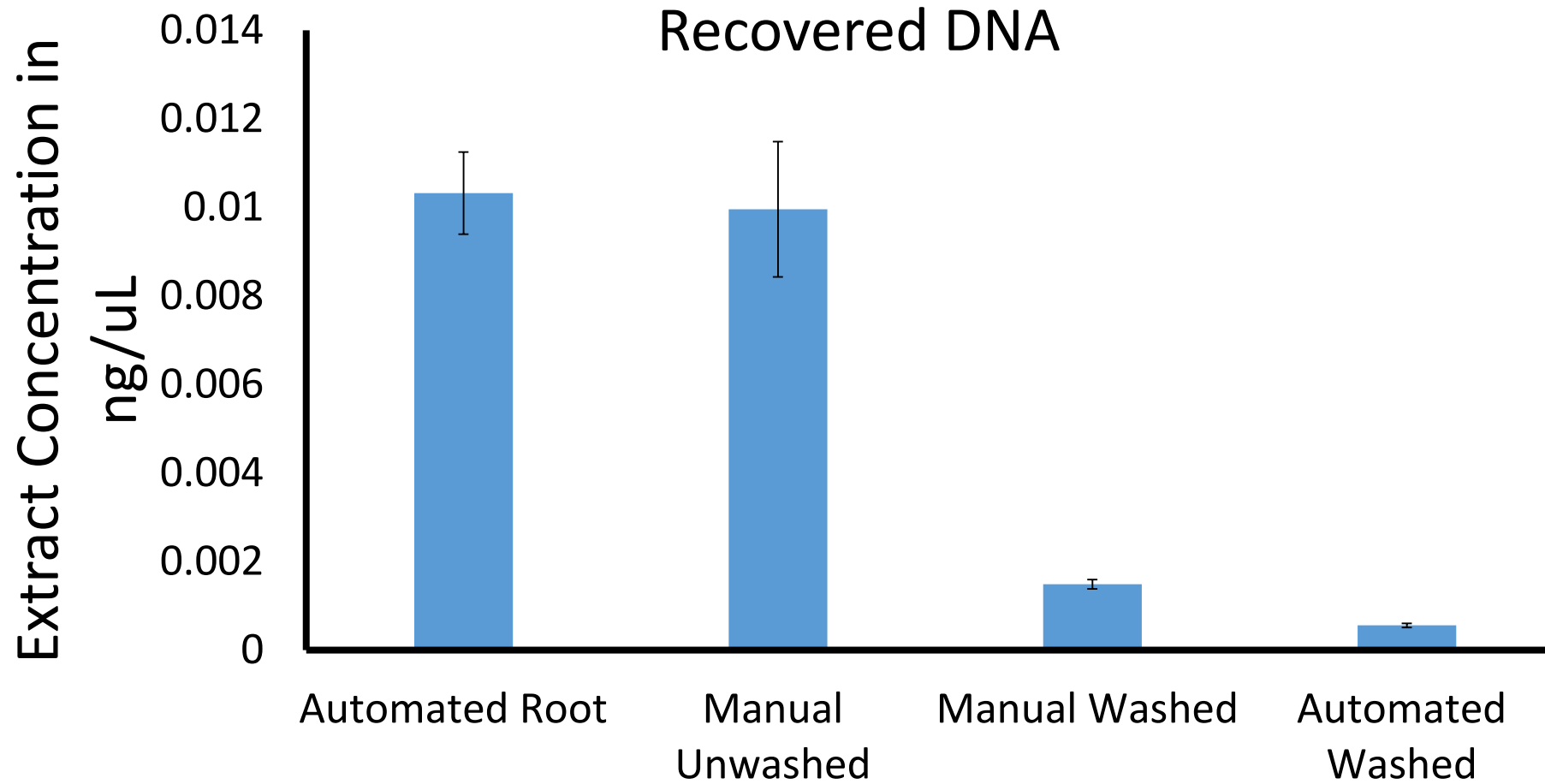
Innotyper 21

- 20 Retrotransposon Insertion Polymorphism (RIP)
 - Amelogenin
- 21 Total

ForenSeq Signature Prep Mix B

- 27 Autosomal STRs
 - 24 Y-STRs, 7 X-STRs
 - 94 Identity SNPs
 - 78 piSNPs
- >200 Total Markers

Quantification

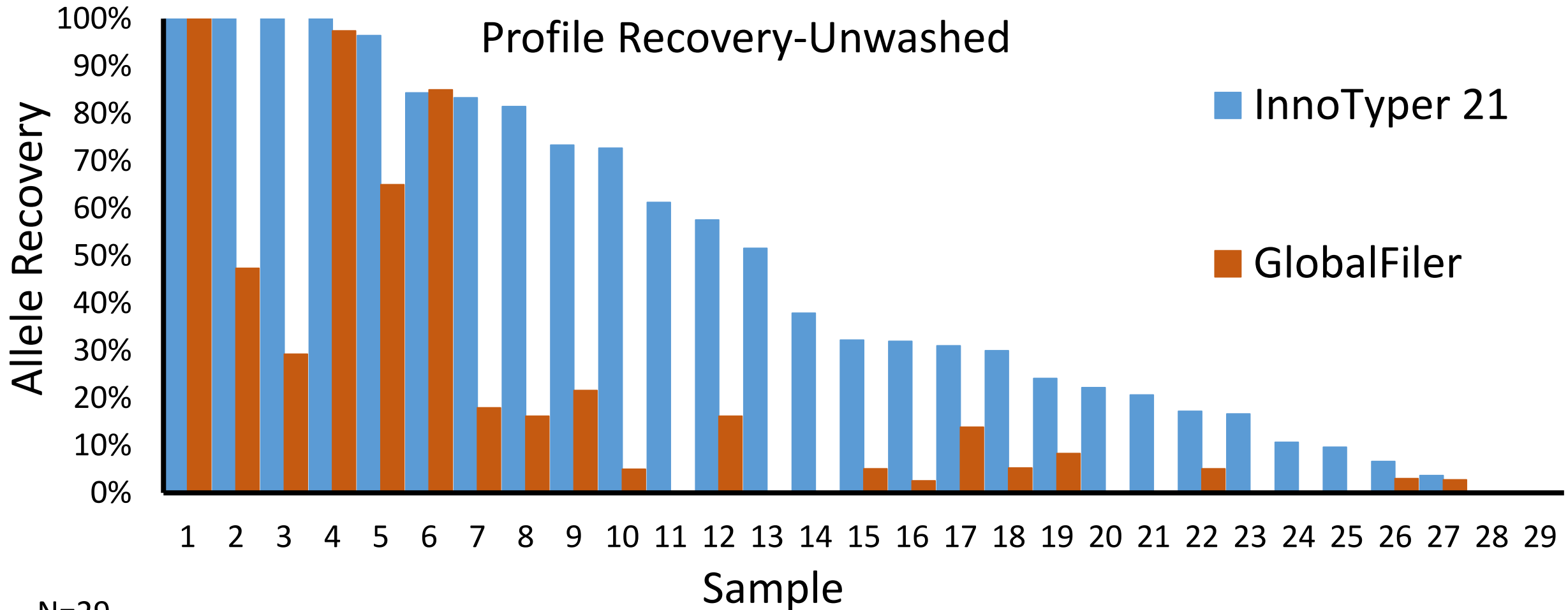


Samples quantified with Quantiplex Pro short target amplicon



Capillary Electrophoresis Results

Unwashed Rootless Hair

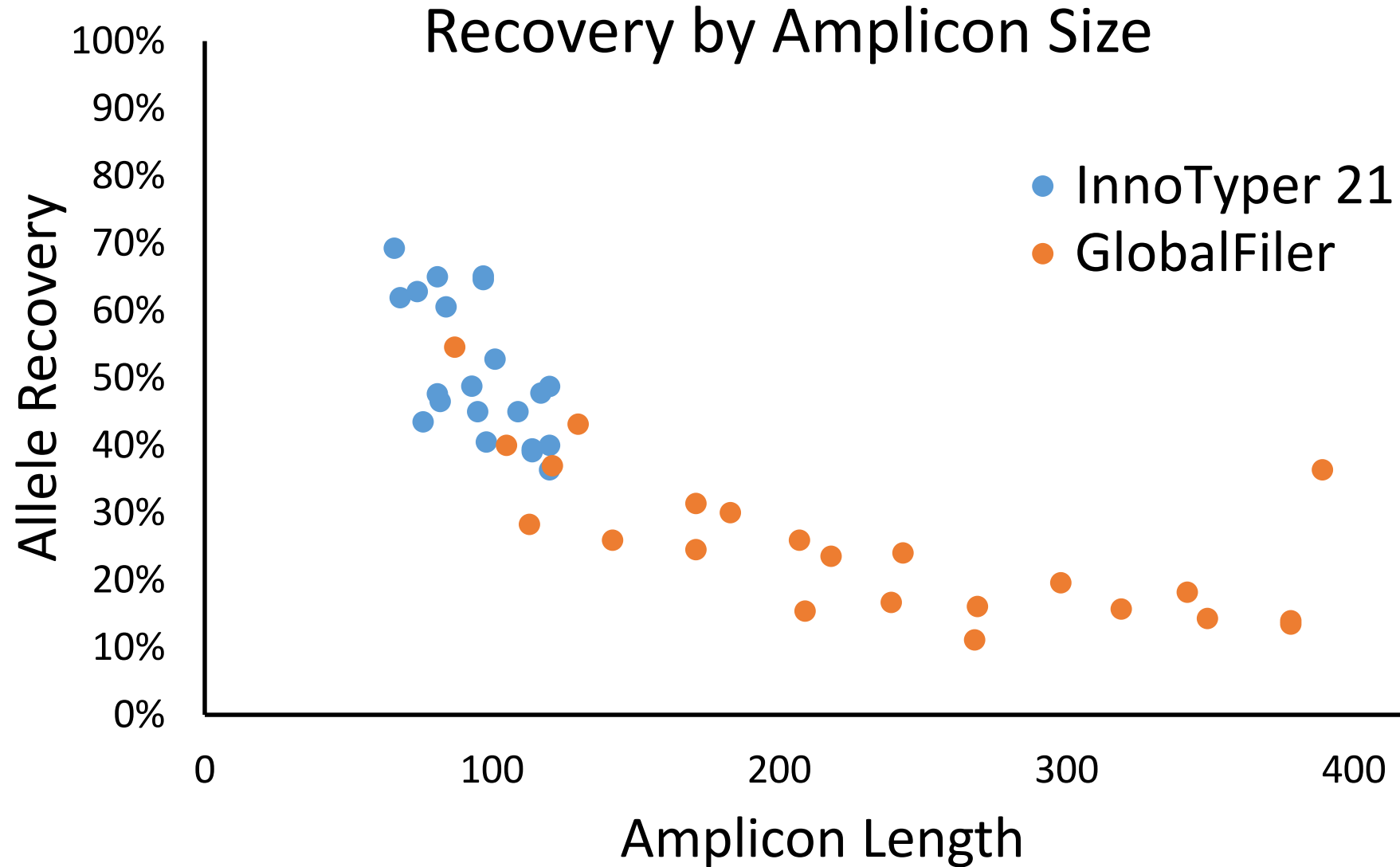


N=29

InnoTyper Average Allele recovery= 50.2%, 7 drop in alleles

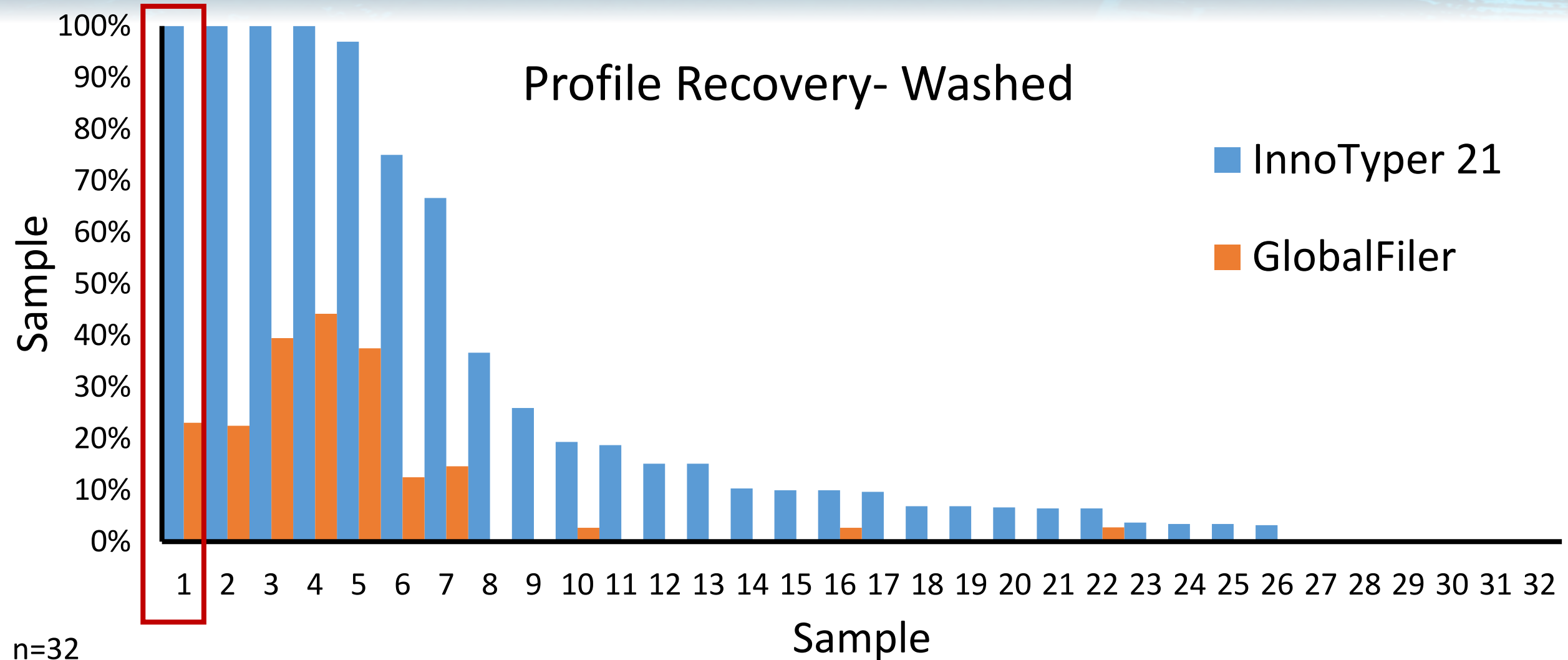
GlobalFiler Average Allele recovery= 22.3%, 2 drop in alleles

Unwashed Rootless Hair: GlobalFiler and InnoTyper 21



Washed Rootless Hair

Profile Recovery- Washed

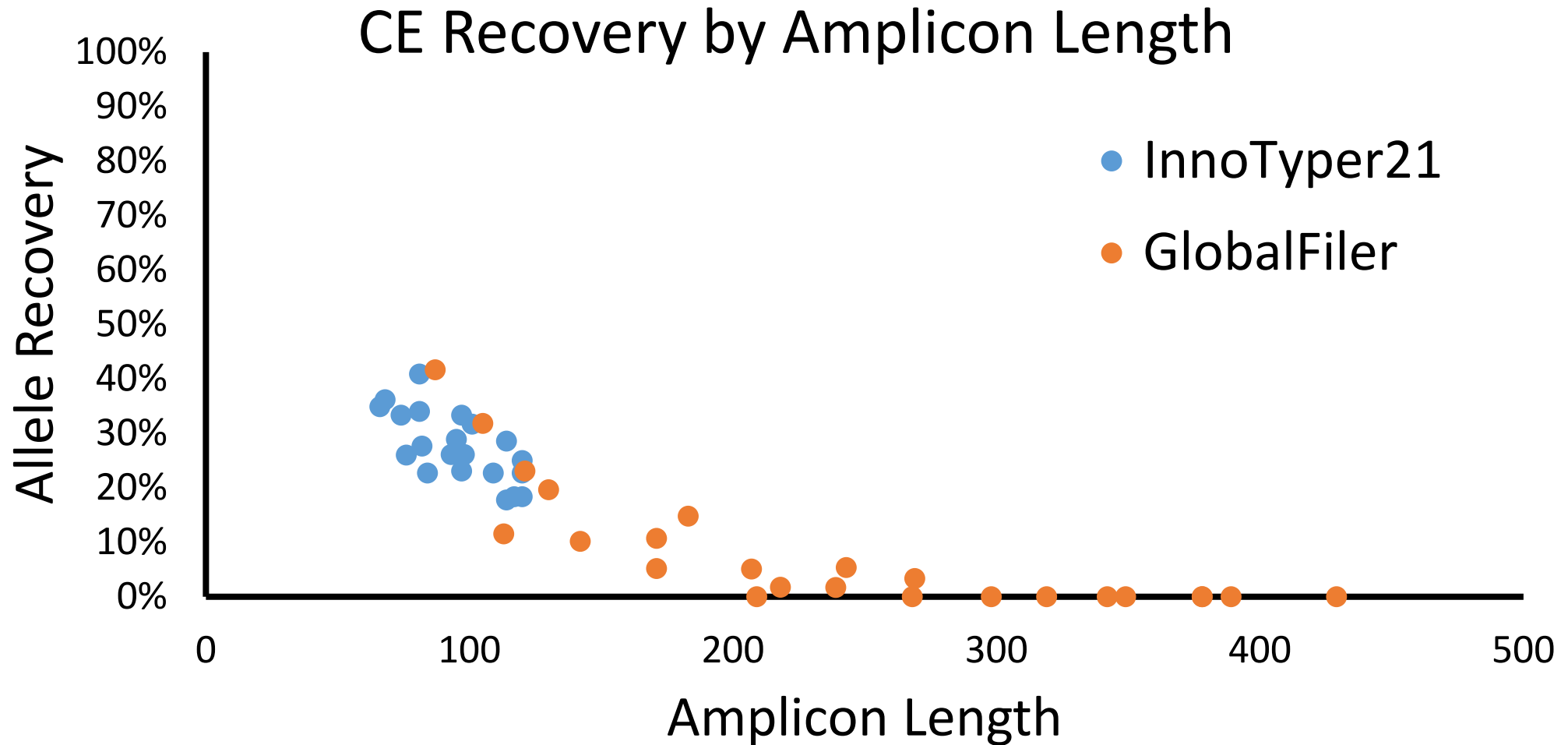


n=32

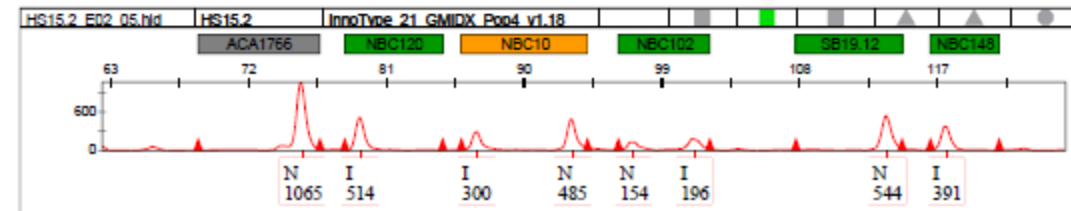
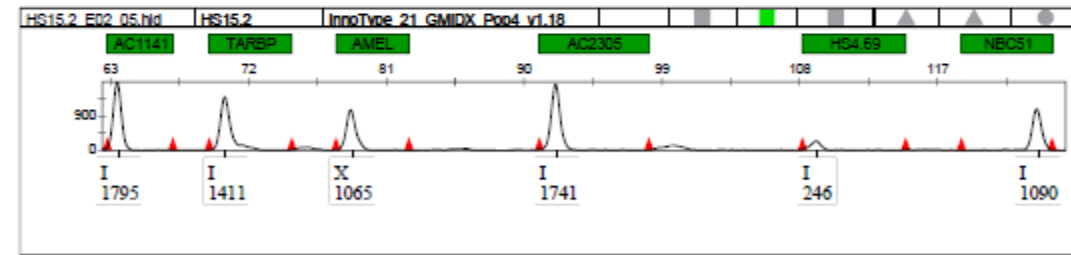
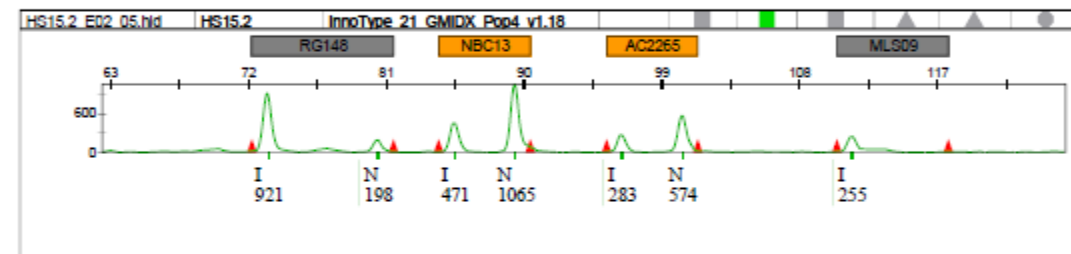
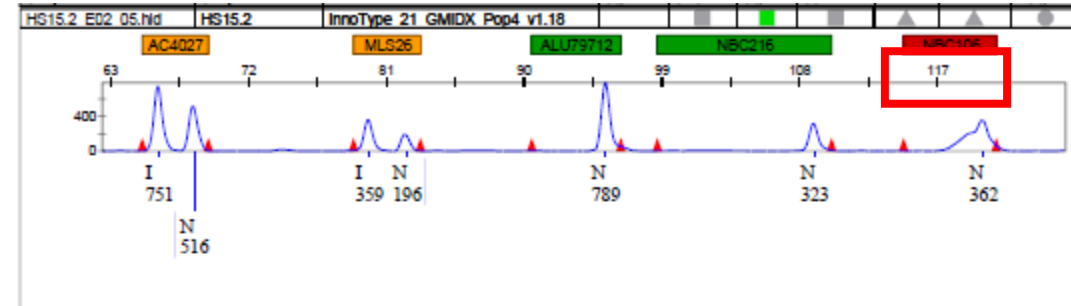
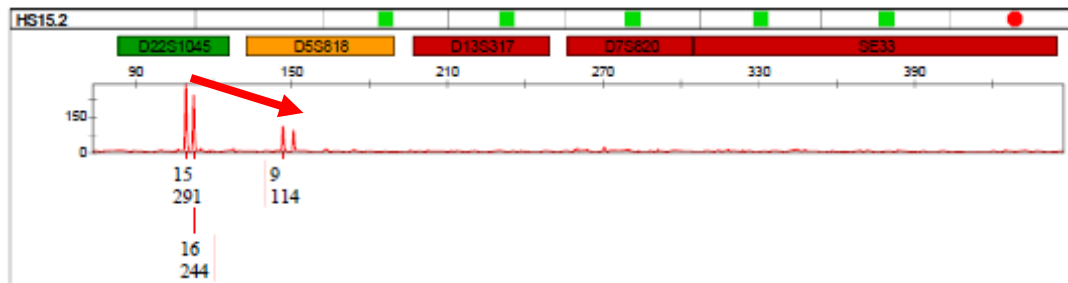
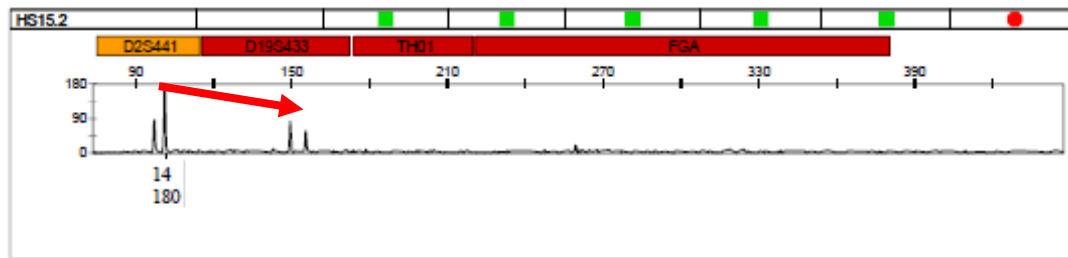
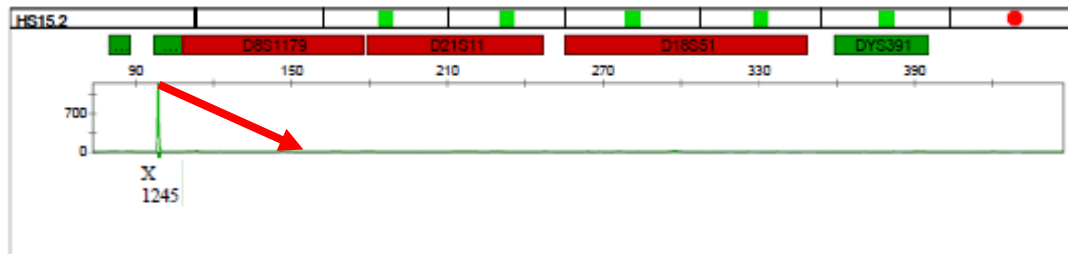
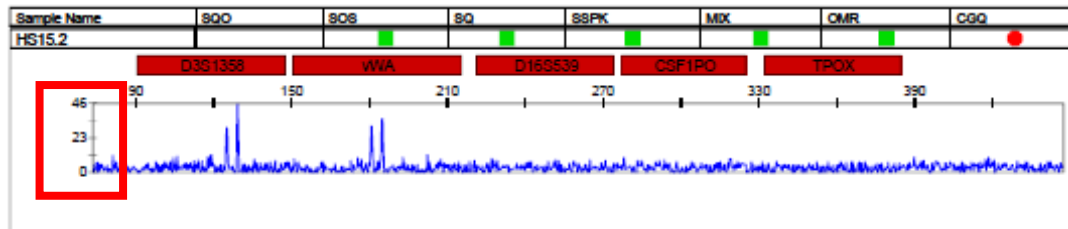
InnoTyper Average Allele Recovery= 26.8%, 4 drop in alleles; profiles with less than 25% recovery


GlobalFiler Average Allele Recovery= 6.3%, clean

Washed Rootless Hair: GlobalFiler and InnoTyper 21



Example Electropherogram

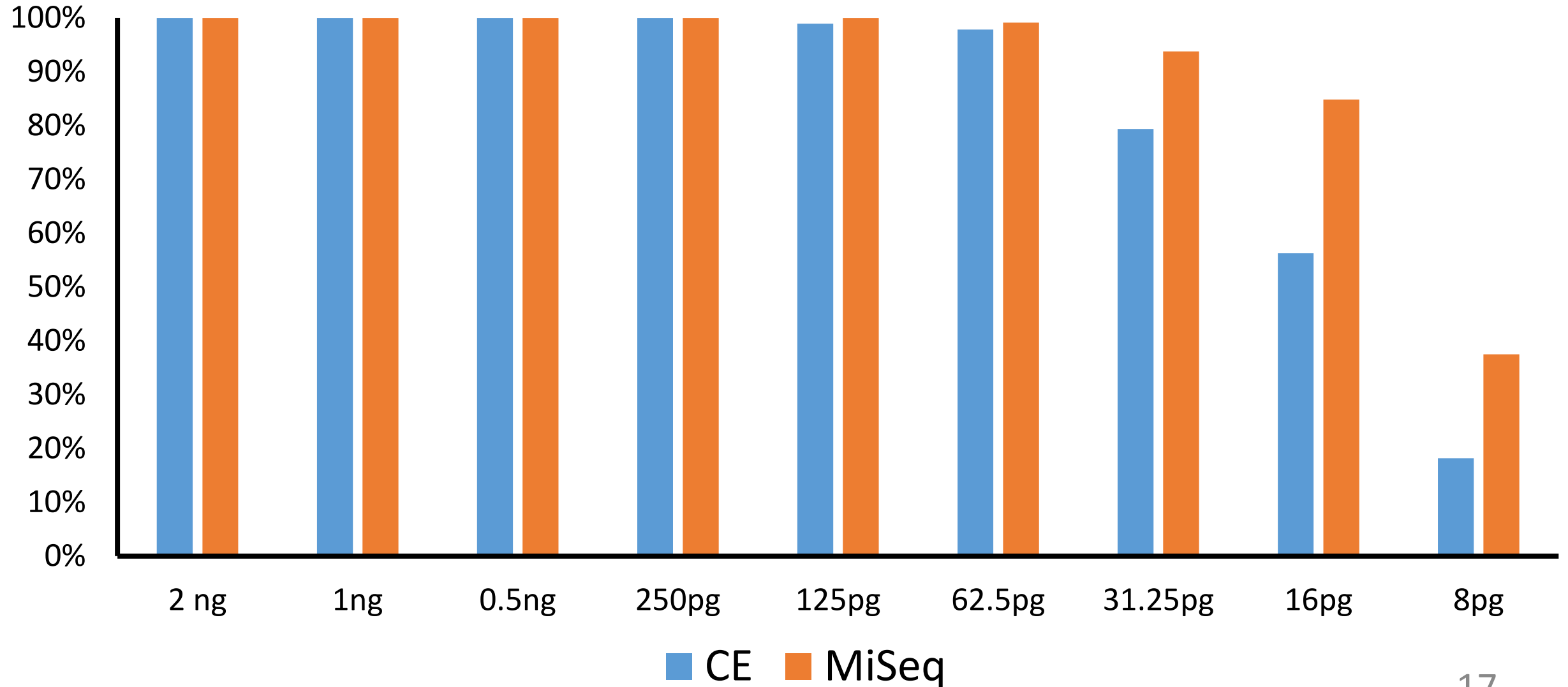




Massively Parallel Sequencing Results

Control Sensitivity GlobalFiler and ForenSeq

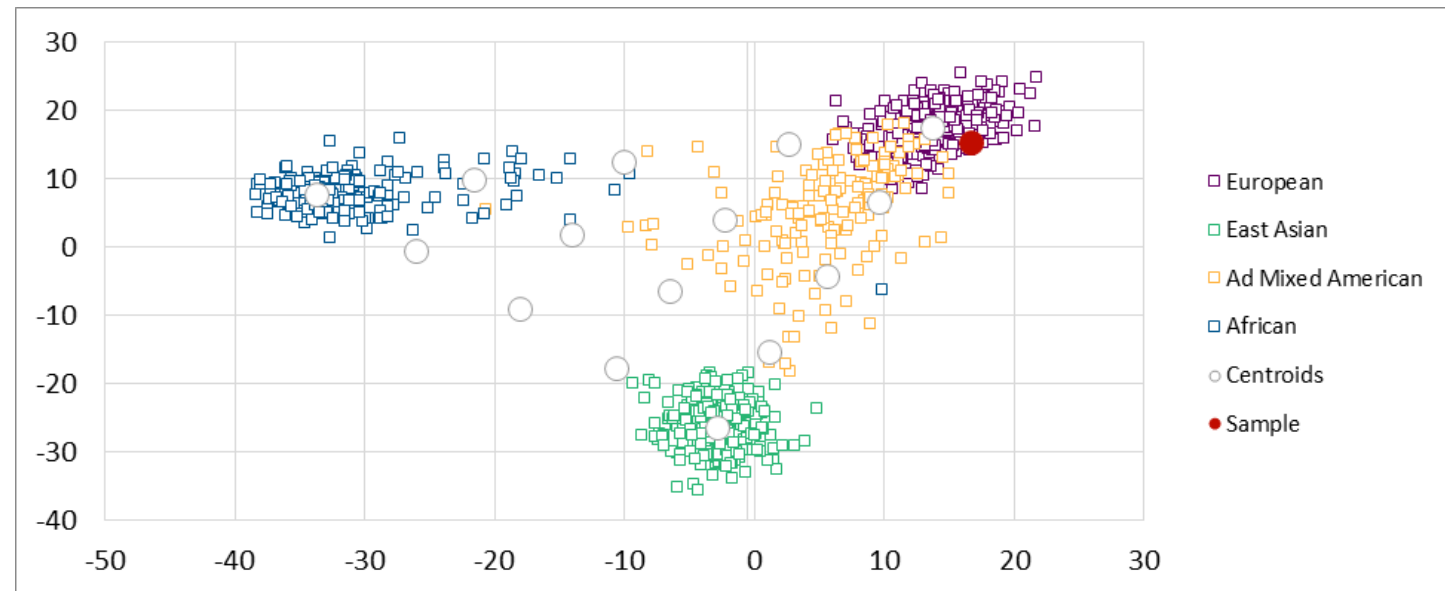
Recovered CODIS STRs



DNA Signature Prep Hair Data

- 5 hairs genotyped
- Successful Ancestry with 4 of 4 self declared samples
- 5 uL of extract for MPS vs. 15 uL of extract for CE

Sample	GF Loci	ForenSeq STR Loci	iSNP Loci	piSNP loci
HS5	8	25	70	58
HS15	7	15	50	38
HS21	11	8	80	63
HS29	8	16	77	67
HS37	4	8	41	30



DNA Signature Prep Hair Data

Sample Comparison

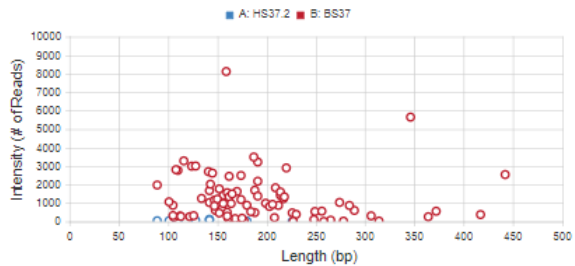
(Sample A)
HS37.2
3.0 - Lucas HRX_2

vs.

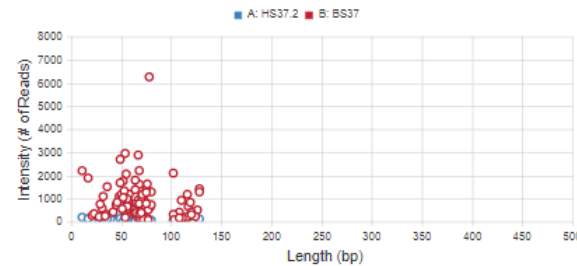
(Sample B)
BS37
3.0 - Lucas HRX_2
Lucas Grant

CHOOSE SAMPLE

STRs

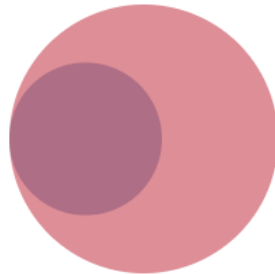


IDENTITY SNPs



LOCI AVAILABLE FOR COMPARISON

HS37.2
49 Loci



BS37
152 Loci

Intersecting Loci: 49

DISCORDANCE

5 STRs

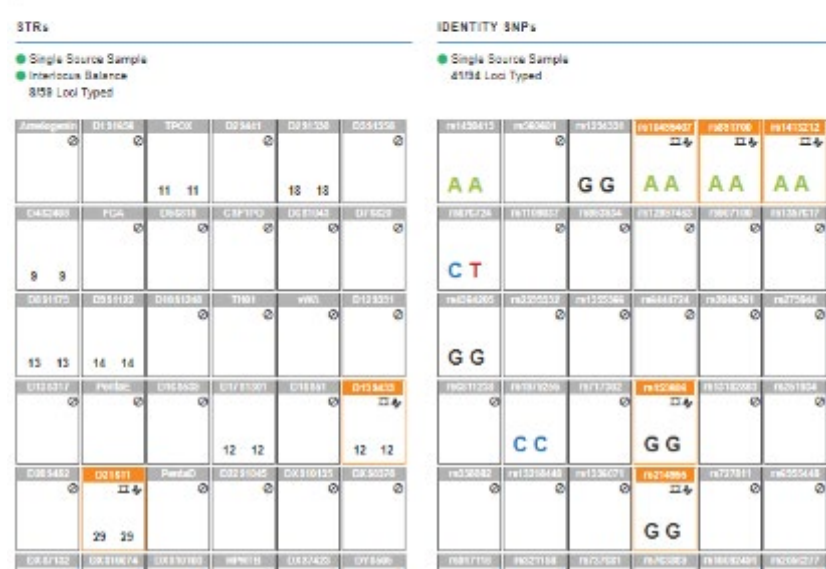
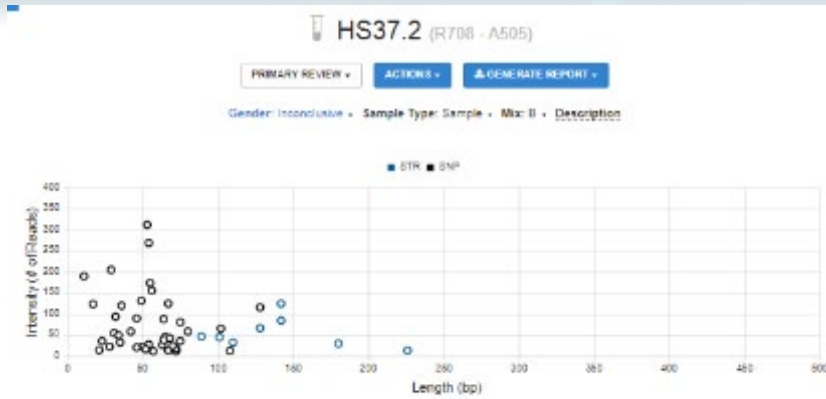
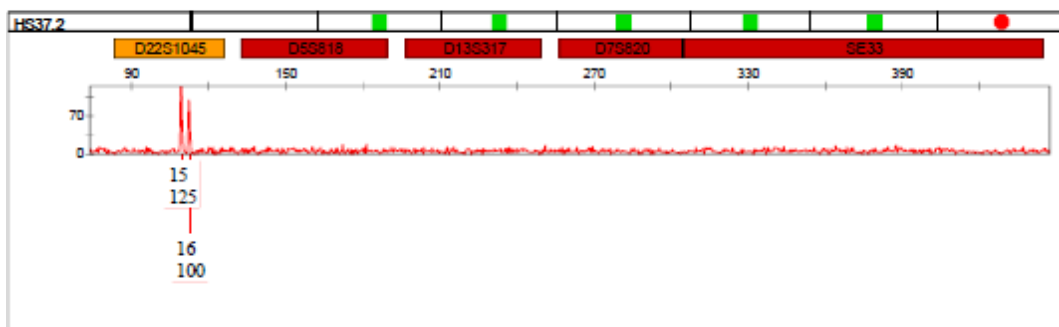
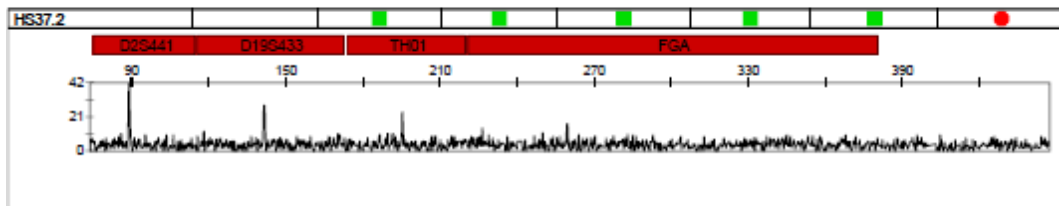
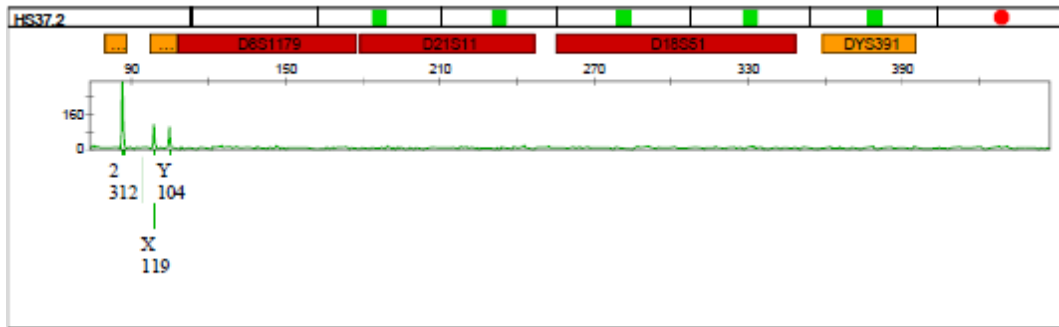
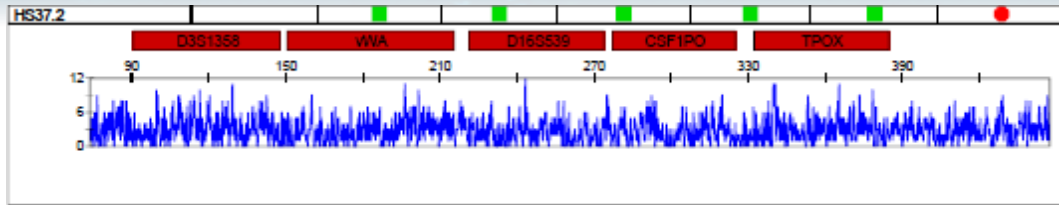
D2 S1338	D4 S2408	D9 S1122	D19 S433	D21 S11
18 18	9 9	14 14	12 12	29 29
16 18	9 10	13 14	12 13	29 30

16 IDENTITY SNPs

rs1254331 G G	rs891700 A A	rs1413212 A A	rs763969 C C	rs4606077 C C	rs10776839 G G
G A	A G	A G	C T	T C	G T
rs954681 T T	rs2107612 A A	rs1335879 T T	rs1886510 C C	rs722250 G G	rs8078417 T T
T C	G A	A T	C T	C G	C T
rs1433232 A A	rs445251 C C	rs722058 A A	rs221956 T T		
C A	C G	A G	T C		

- Concordance between washed hair shaft sample and reference buccal profile
- Any discordance is caused by allele drop out

Example Profile



- GlobalFiler- 6 alleles
- Amelogenin
 - Y-Indel
 - 2 Autosomal STR Loci

- ForenSeq Signature Prep
85 alleles
- 8 Autosomal STR Loci
 - 41 iSNP Loci
 - 30 piSNP Loci

Conclusions

- Novel extraction chemistries allow collection of highly degraded but useful nuclear DNA from rootless hair shafts
- DNA collected in this fashion can be typed with amplicons designed smaller than 147 base pairs
- Existing CE assays based on alternative markers allow genotyping of these samples in some cases
- Due to a relaxation of chemistry constraints MPS platforms allow for successful genotyping of rootless hair shafts in some cases
- Alternative methods have increased discriminatory power over mitochondrial DNA haplotypes

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Questions?

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