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

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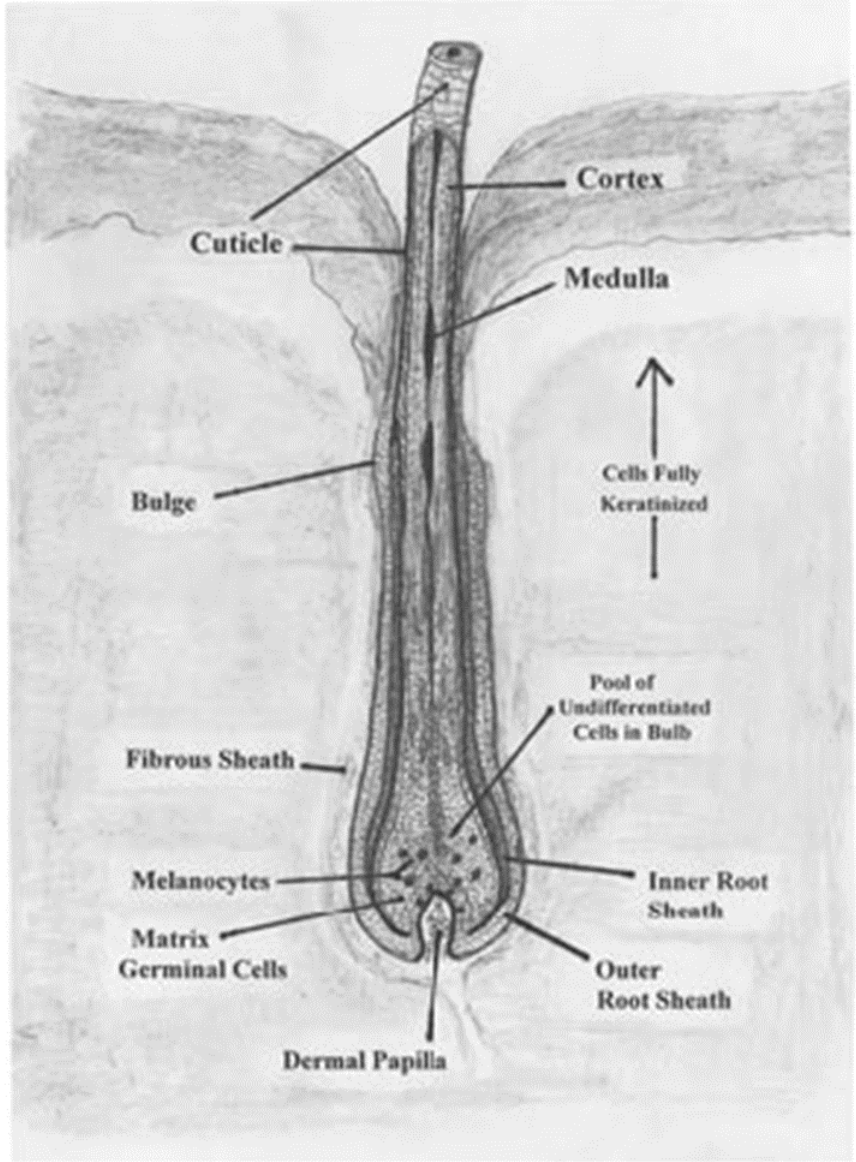
Limitations of Genetic Testing

Challenges commonly seen in biological samples

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

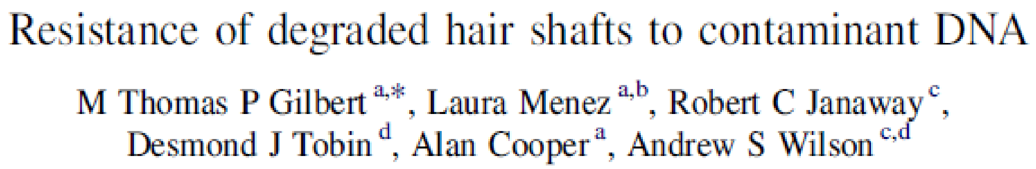


C.A. Linch, D.A. Whiting, M.M. Holland, Human Hair Histogenesis for the Mitochondrial DNA Forensic Scientist, ASTM AMERICAN SOCIETY FOR TESTING AND, United States, 2001, p. 844

Introduction Cont.

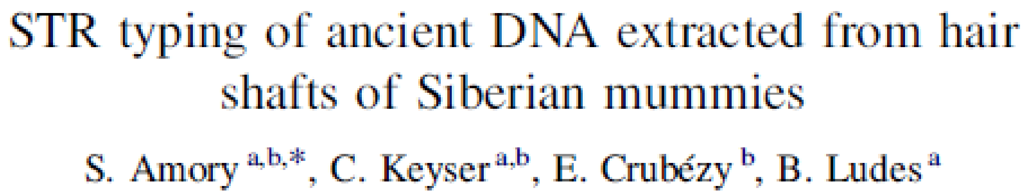
Advantages of Hair

- Persistence
- Can be decontaminated
- Commonly collected as evidence



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]



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]

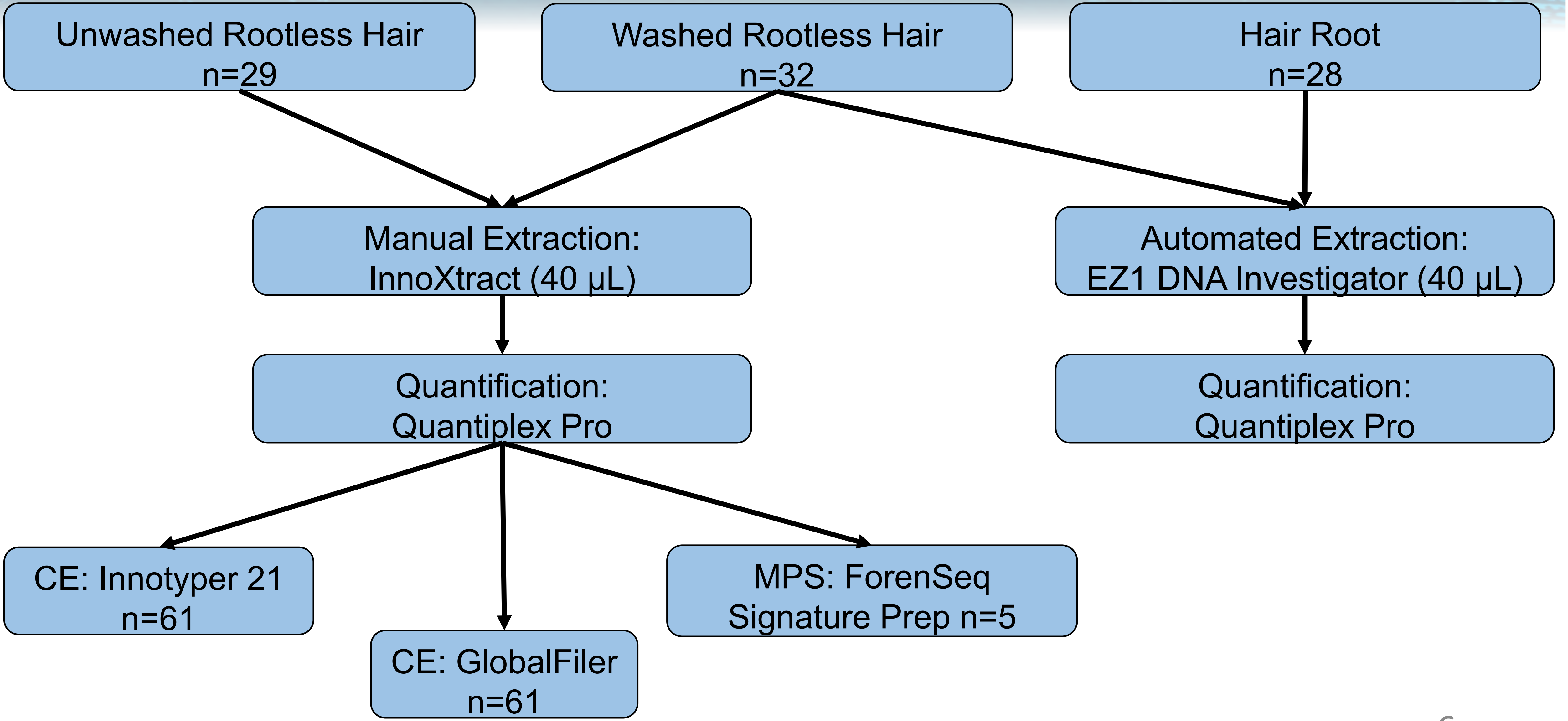
Paleo-Eskimo mtDNA Genome Reveals Matrilineal Discontinuity in Greenland

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]†

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



Unwashed Rootless Hair n=29

Washed Rootless Hair n=32

Hair Root n=28

Manual Extraction: InnoXtract (40 µL)

Automated Extraction: EZ1 DNA Investigator (40 µL)

Quantification: Quantiplex Pro

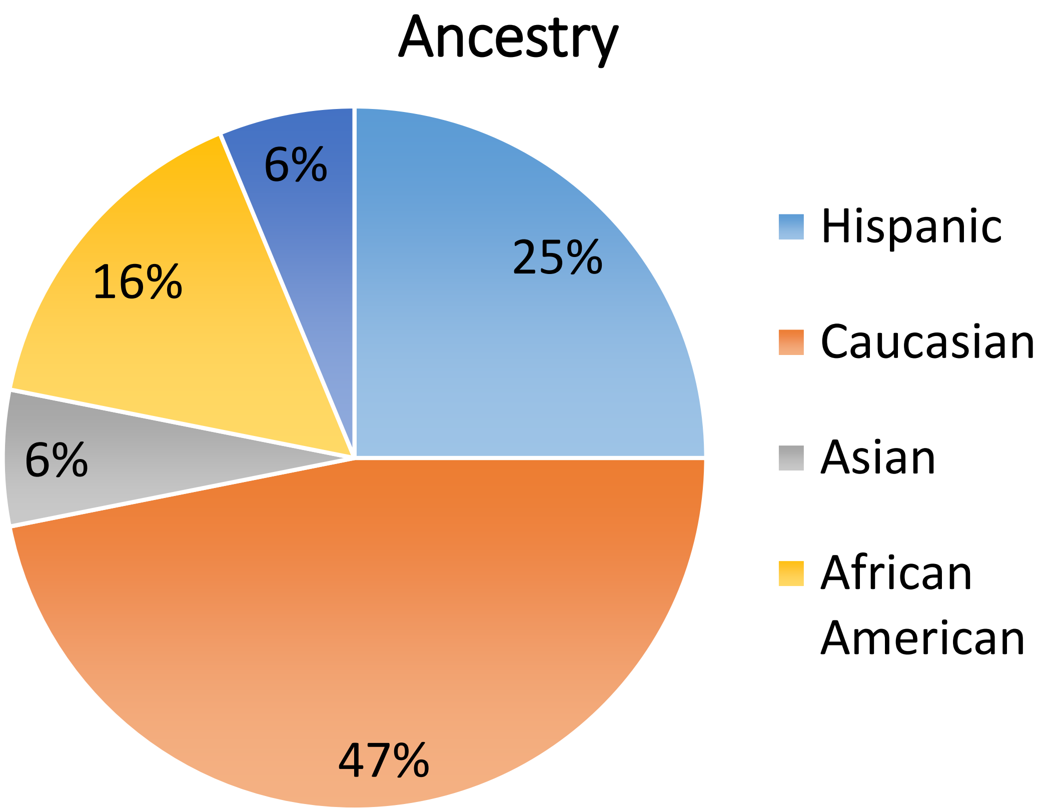
Quantification: Quantiplex Pro

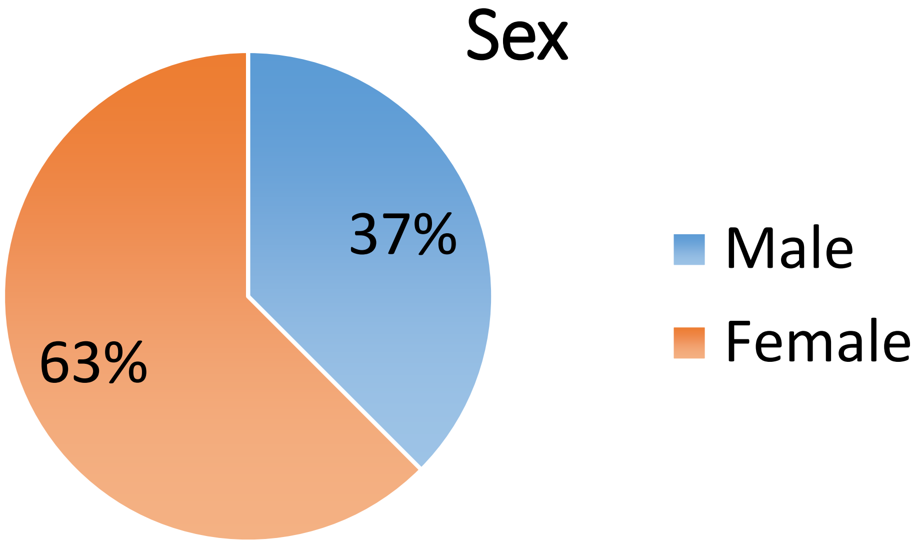
CE: Innotyper 21 n=61

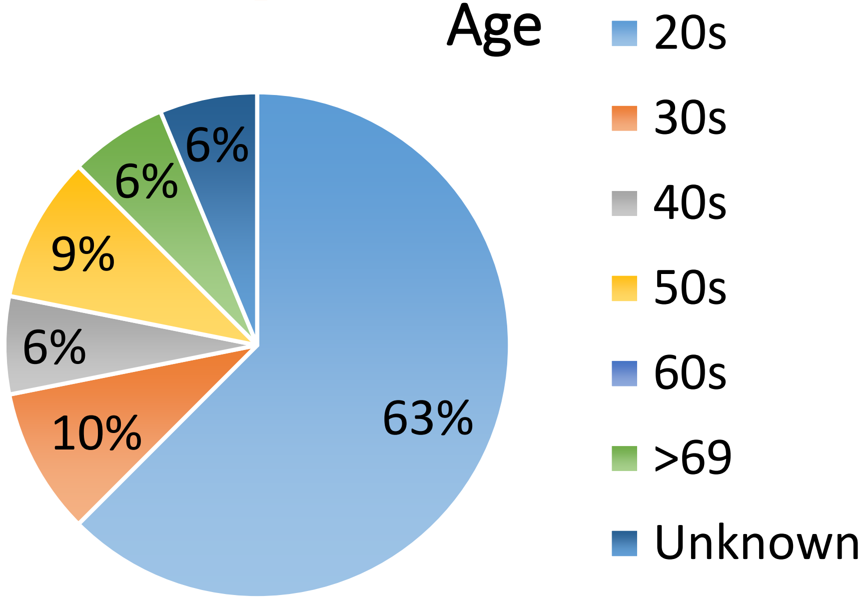
CE: GlobalFiler n=61

MPS: ForenSeq Signature Prep n=5

Sample Population n=32

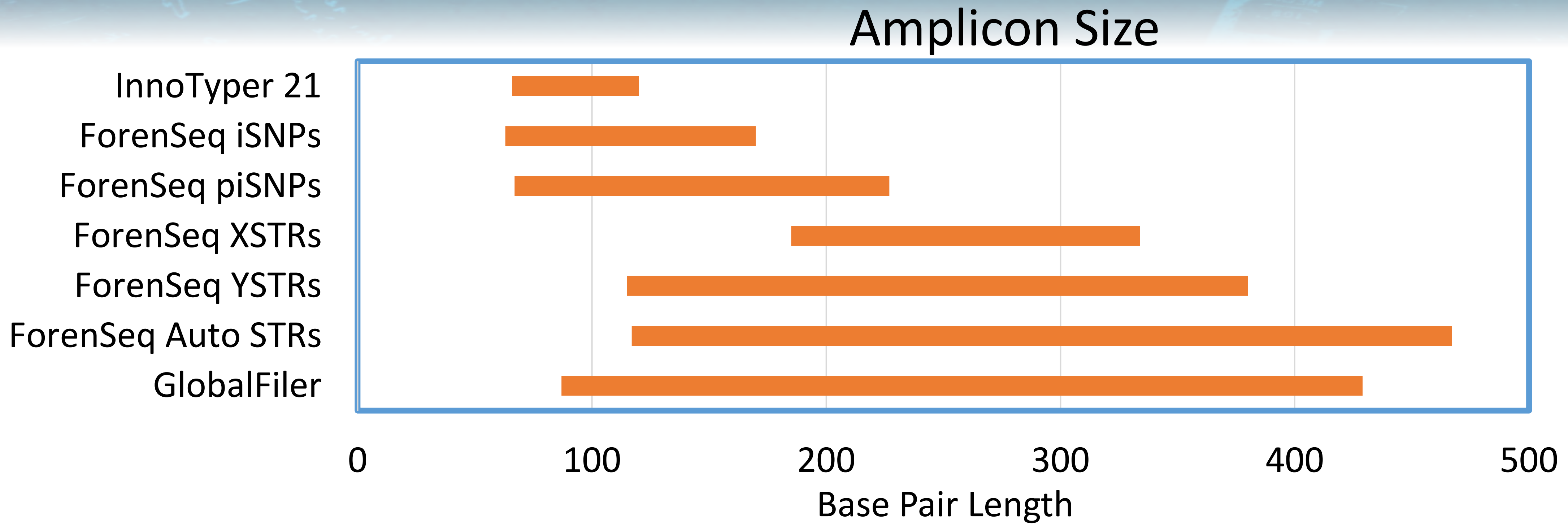






- Hairs collected from personal hair brushes
- Buccal swabs for reference profiles
- Cleaned following adapted Grisedale et. al method

Comparison of Technologies



GlobalFiler
- 21 Autosomal STRs
- DYS319
- Amelogenin
- Y-Indel

24 Total

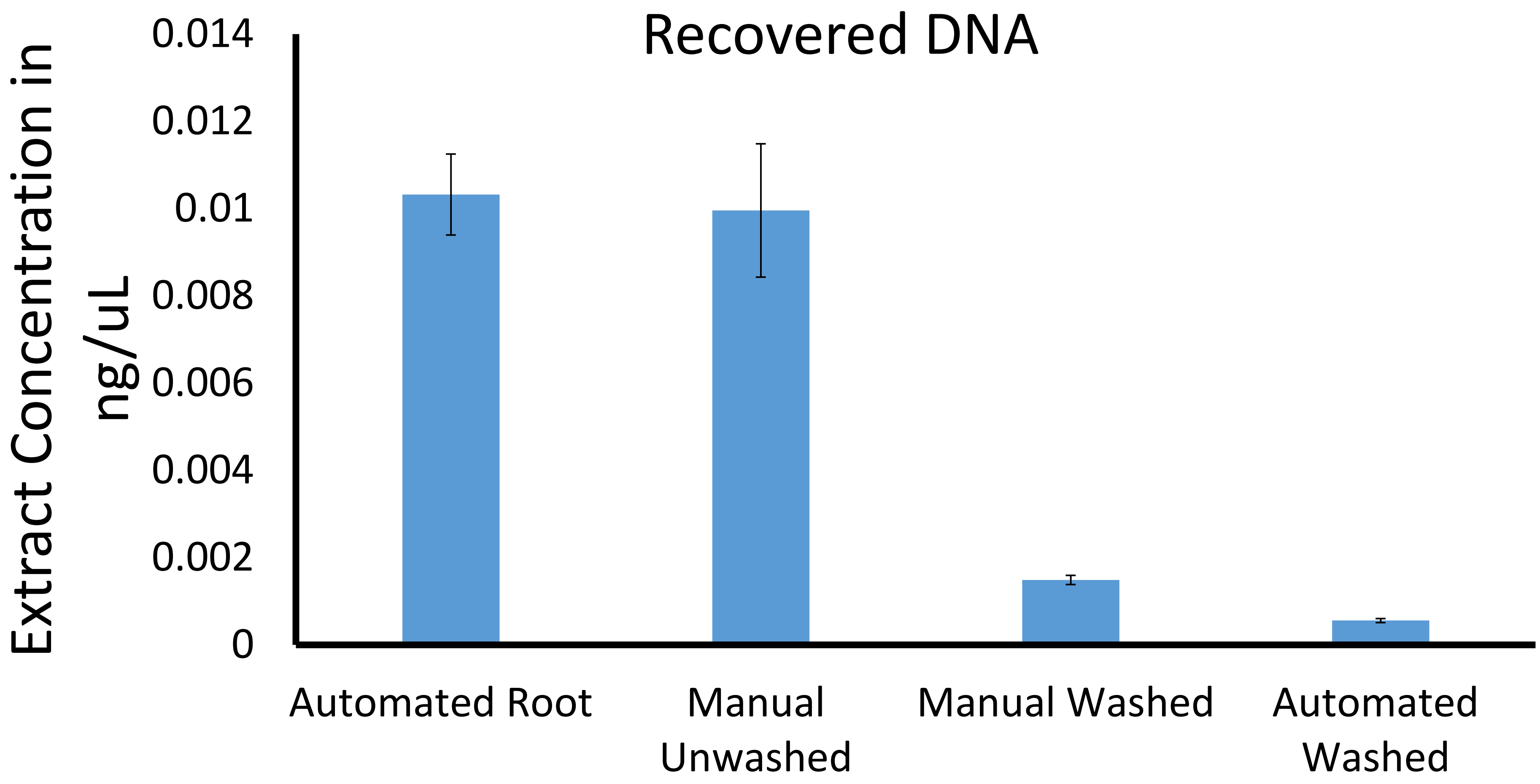
Innotyper 21
- 20 Retrotransposon Insertion Polymorphism (RIP)
- Amelogenin

21 Total

ForenSeq Signature Prep Mix B
- 27 Autosomal STRs
- 24 Y-STRs,7 X-STRs
- 94 Identity SNPs
- 78 piSNPs

>200 Total Markers

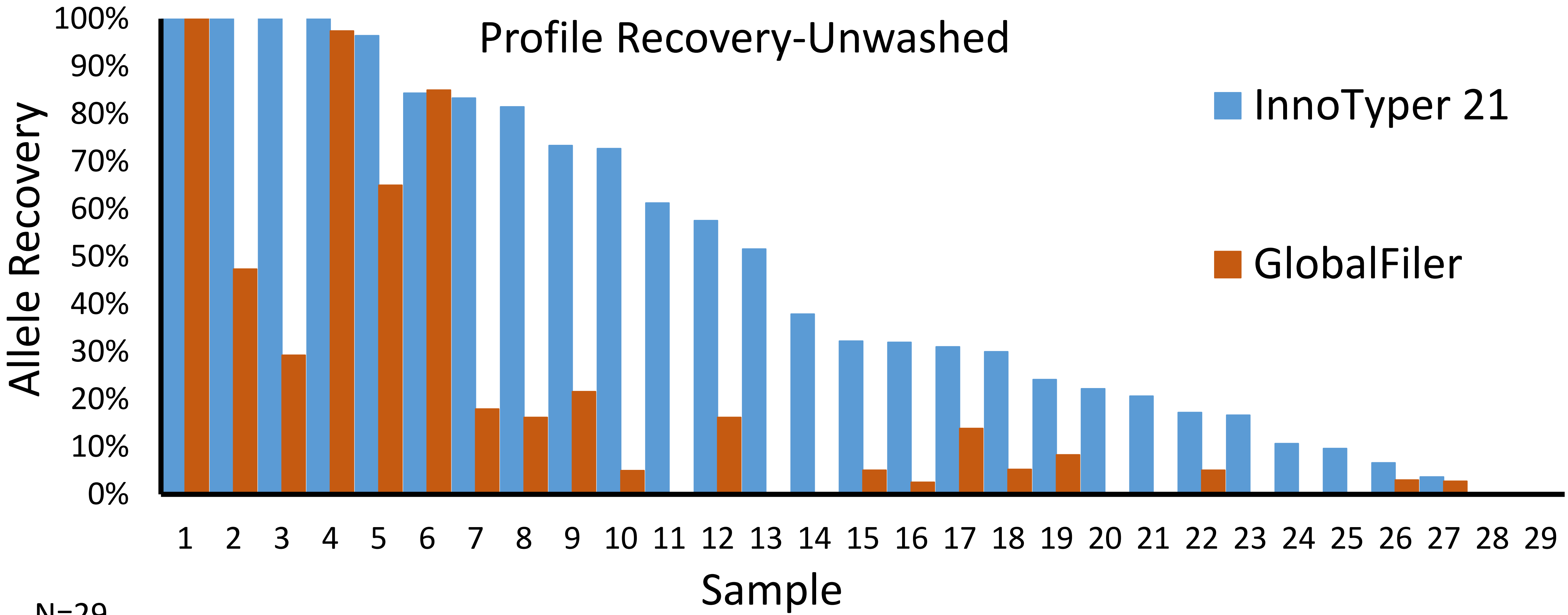
Quantification



Samples quantified with Quantiplex Pro short target amplicon

Capillary Electrophoresis Results

Unwashed Rootless Hair

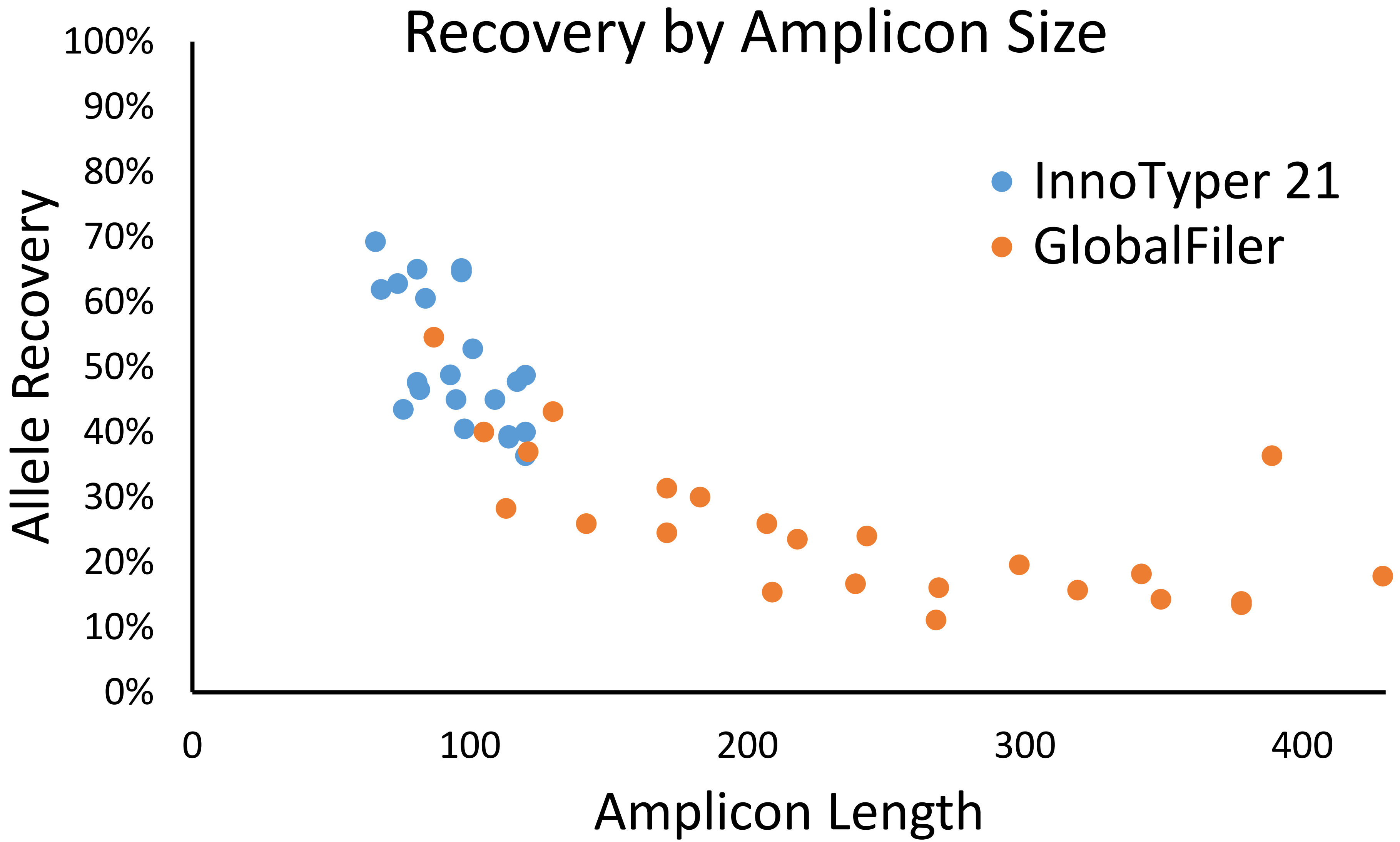


N=29

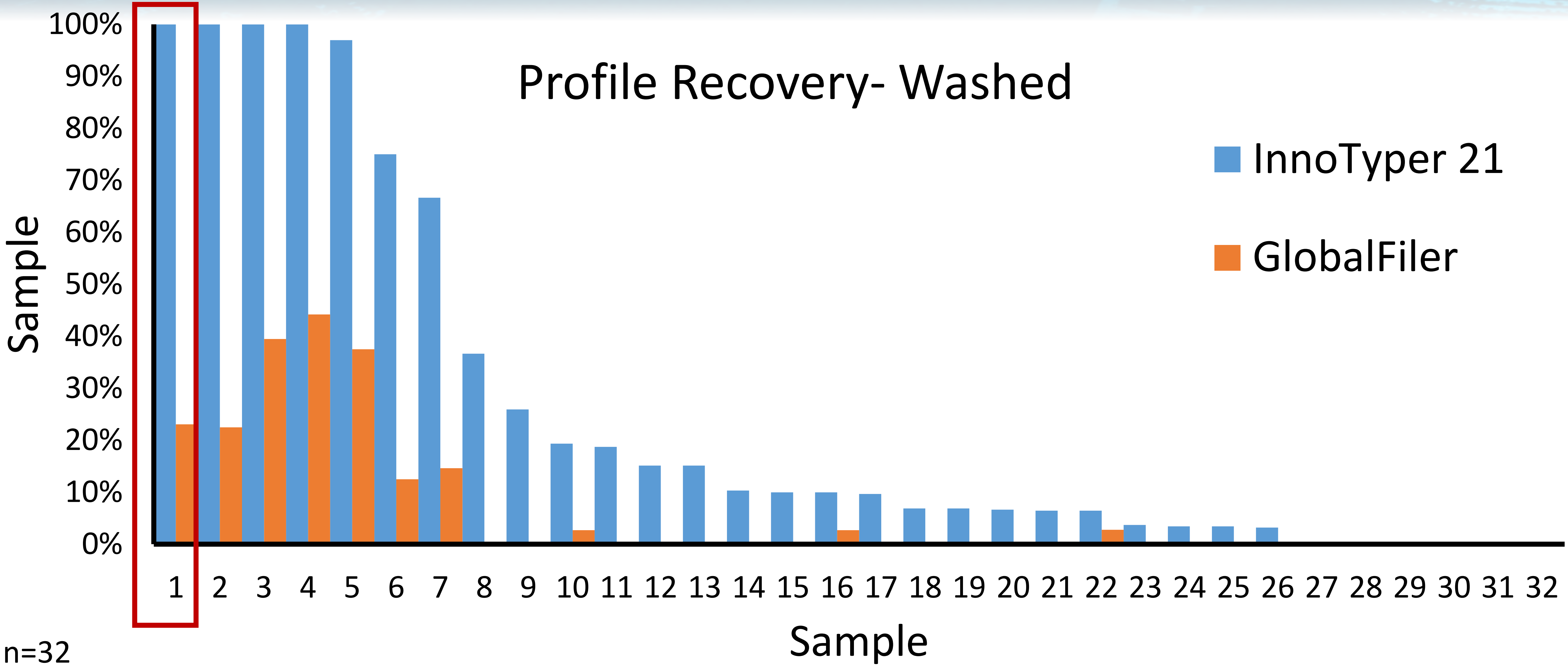
InnoTyper Average Allele recovery= 50.2%, 7 drop in alleles

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

Unwashed Rootless Hair: GlobalFiler and InnoTyper 21



Washed Rootless Hair

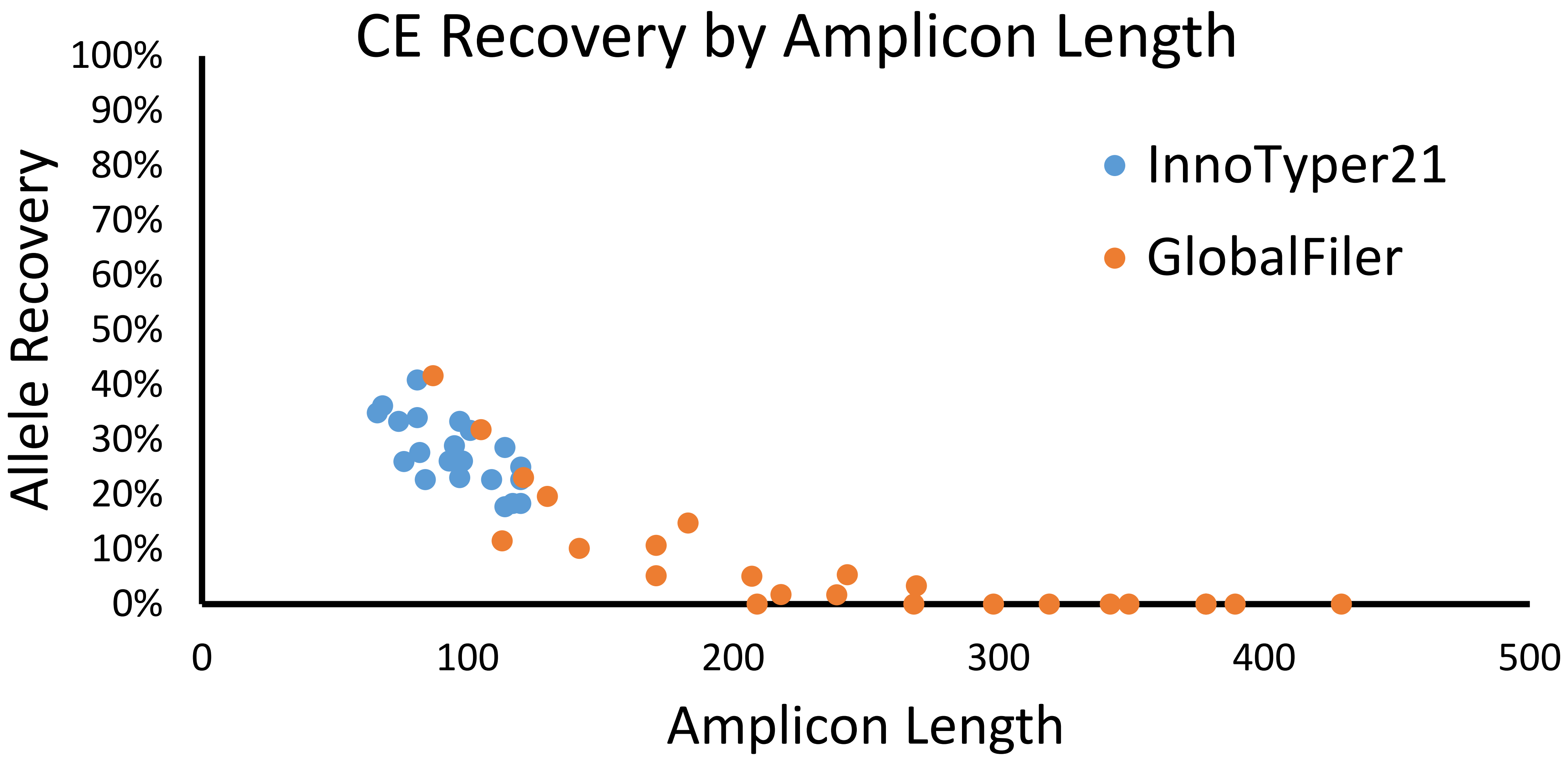


n=32

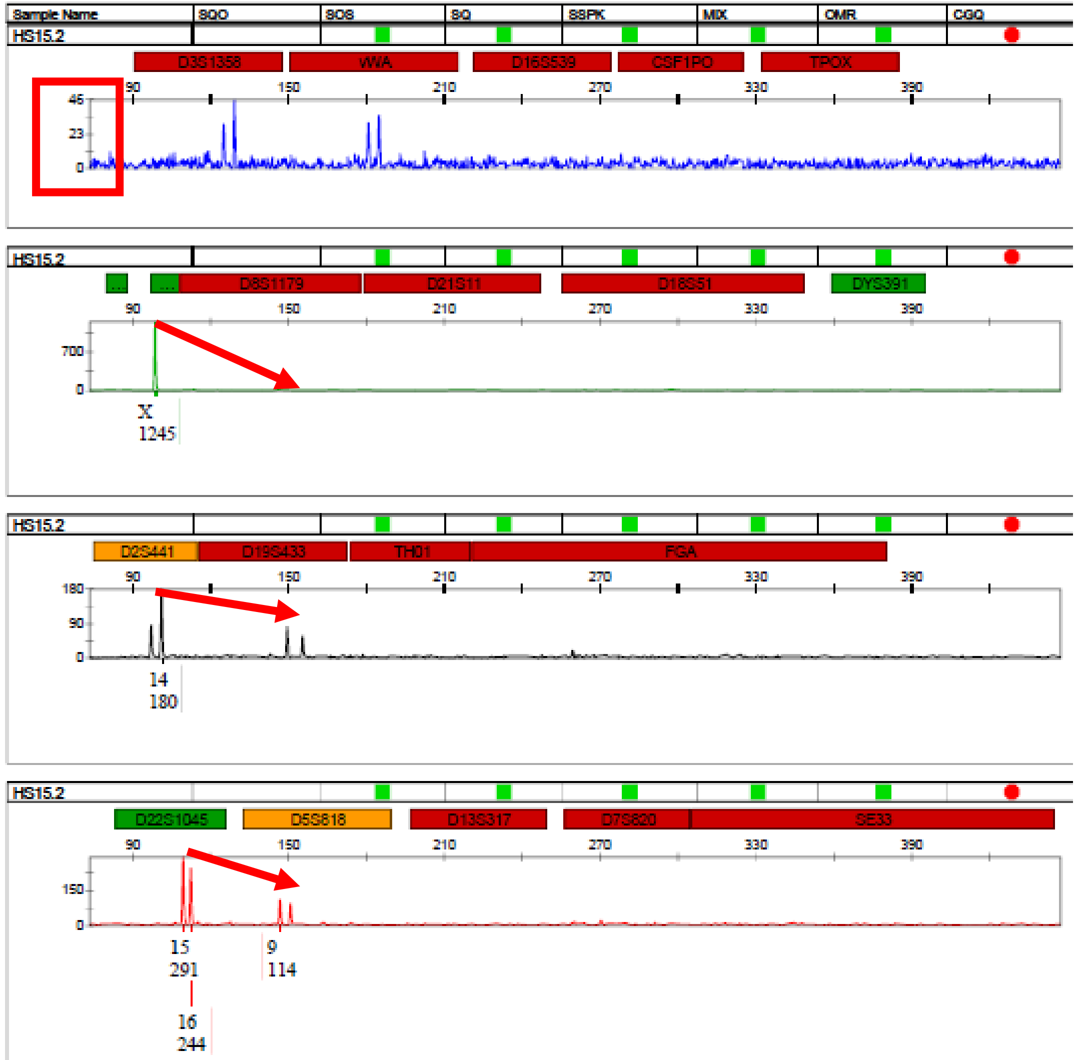
InnoTyper Average Allele Recovery= 26.8%, 4 drop in alleles; profiles with less than 25% recovery

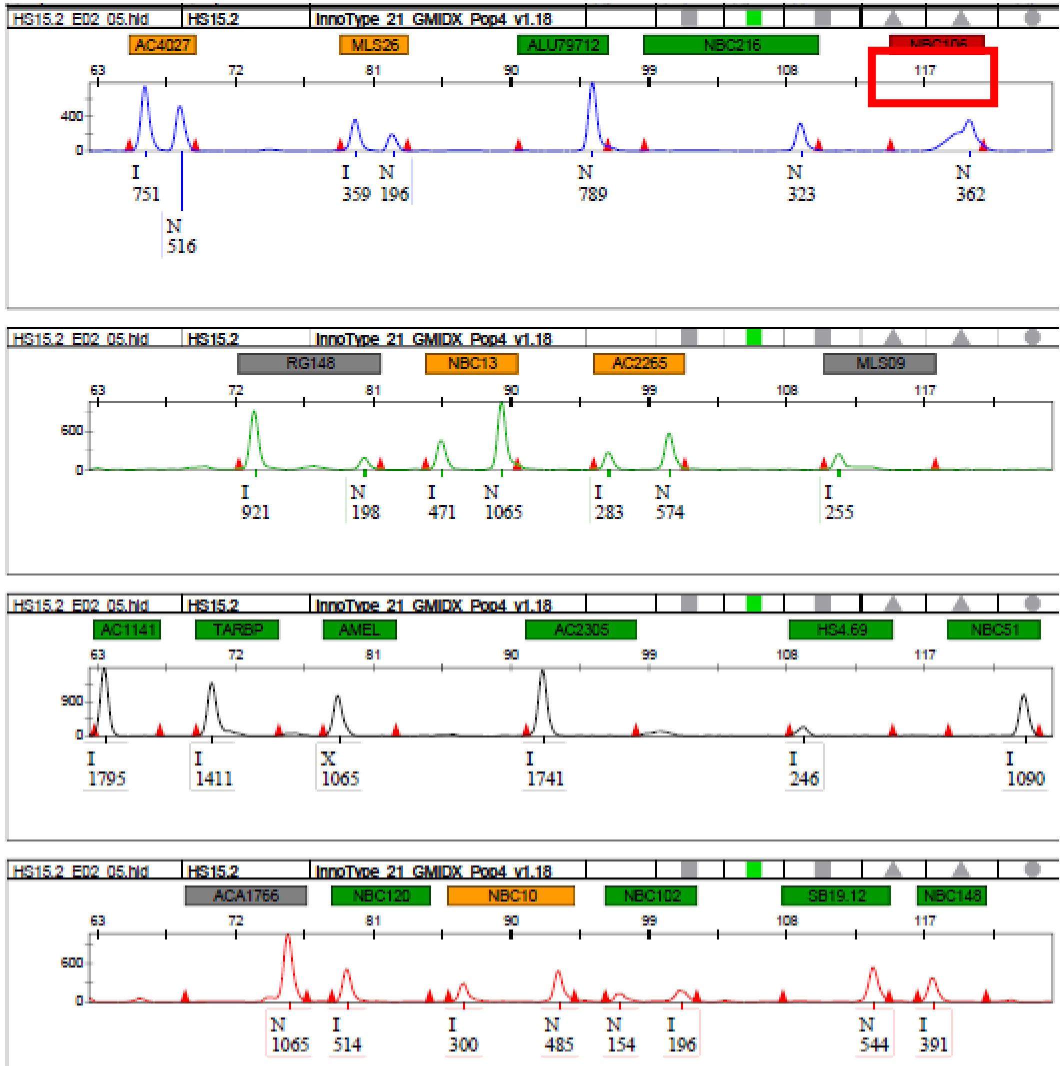
GlobalFiler Average Allele Recovery= 6.3%, clean

Washed Rootless Hair: GlobalFiler and InnoTyper 21



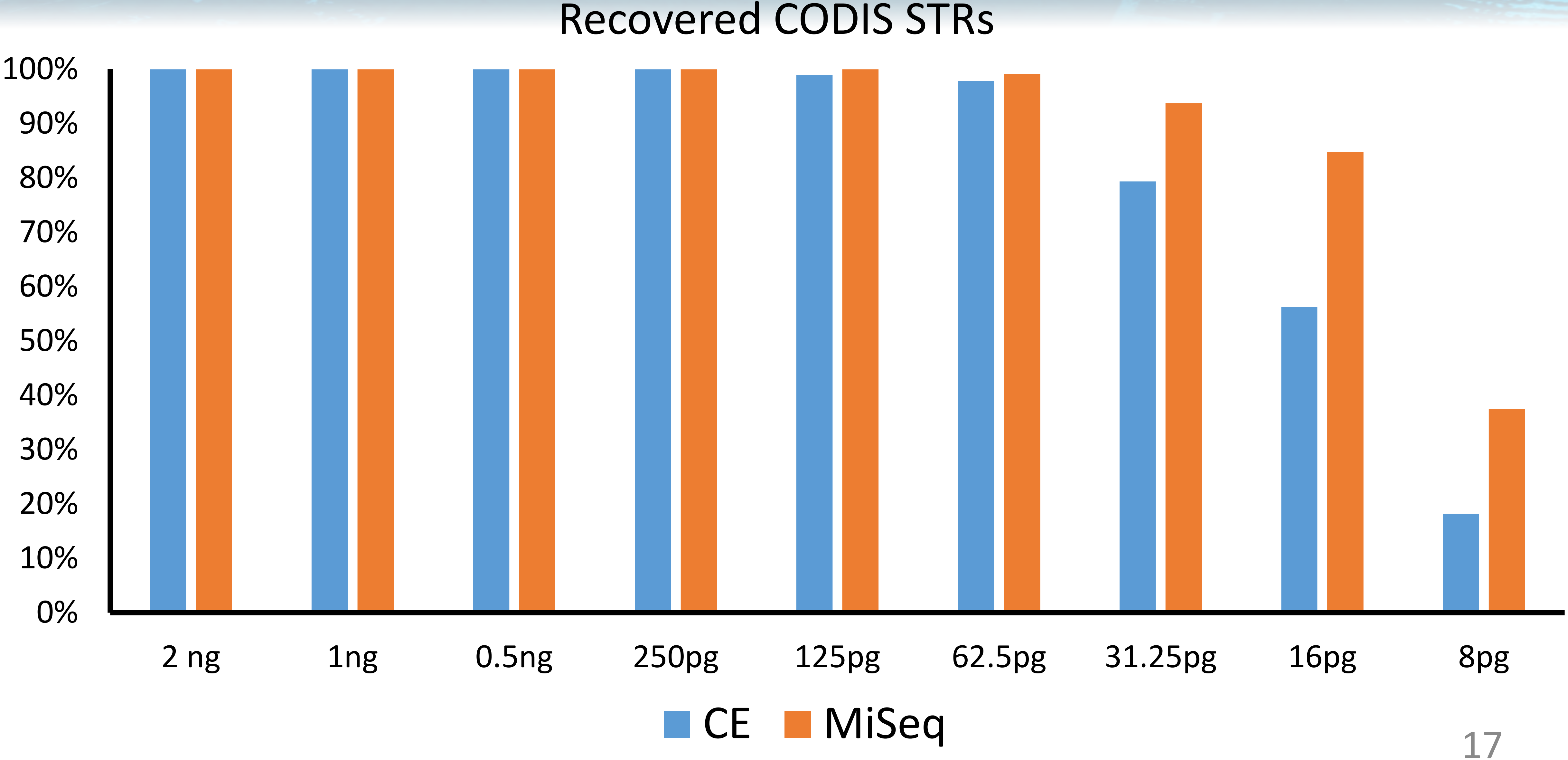
Example Electropherogram





Massively Parallel Sequencing Results

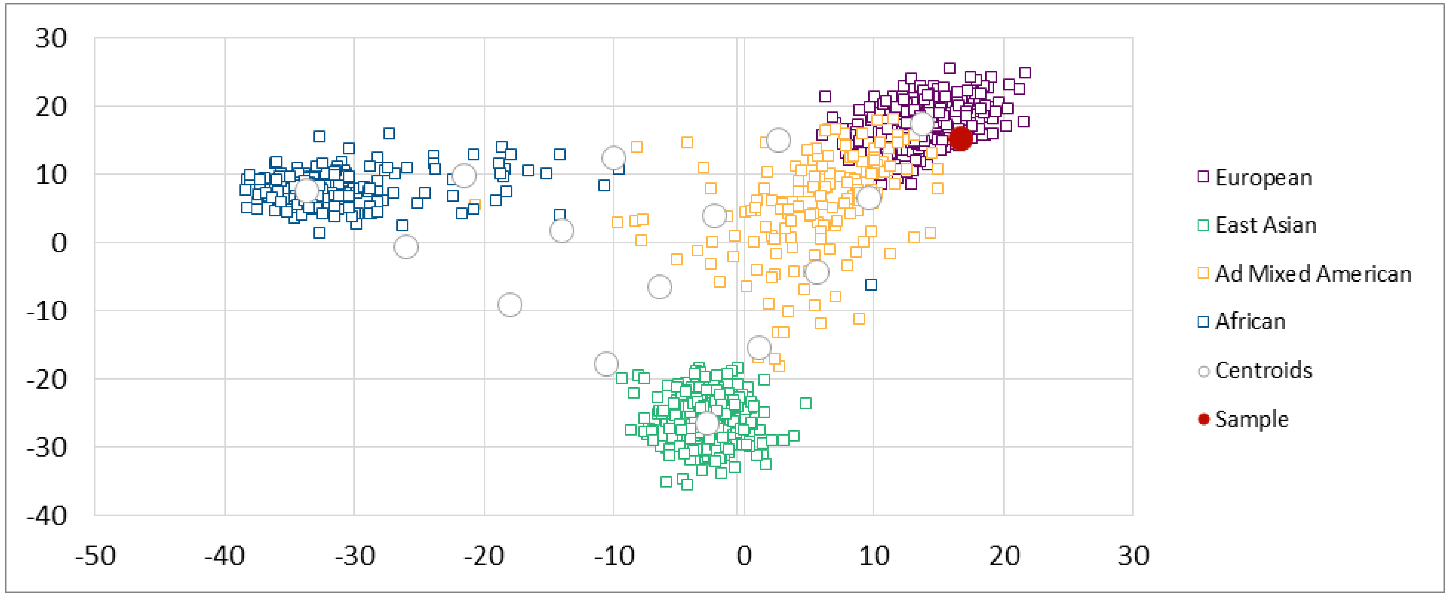
Control Sensitivity GlobalFiler and ForenSeq



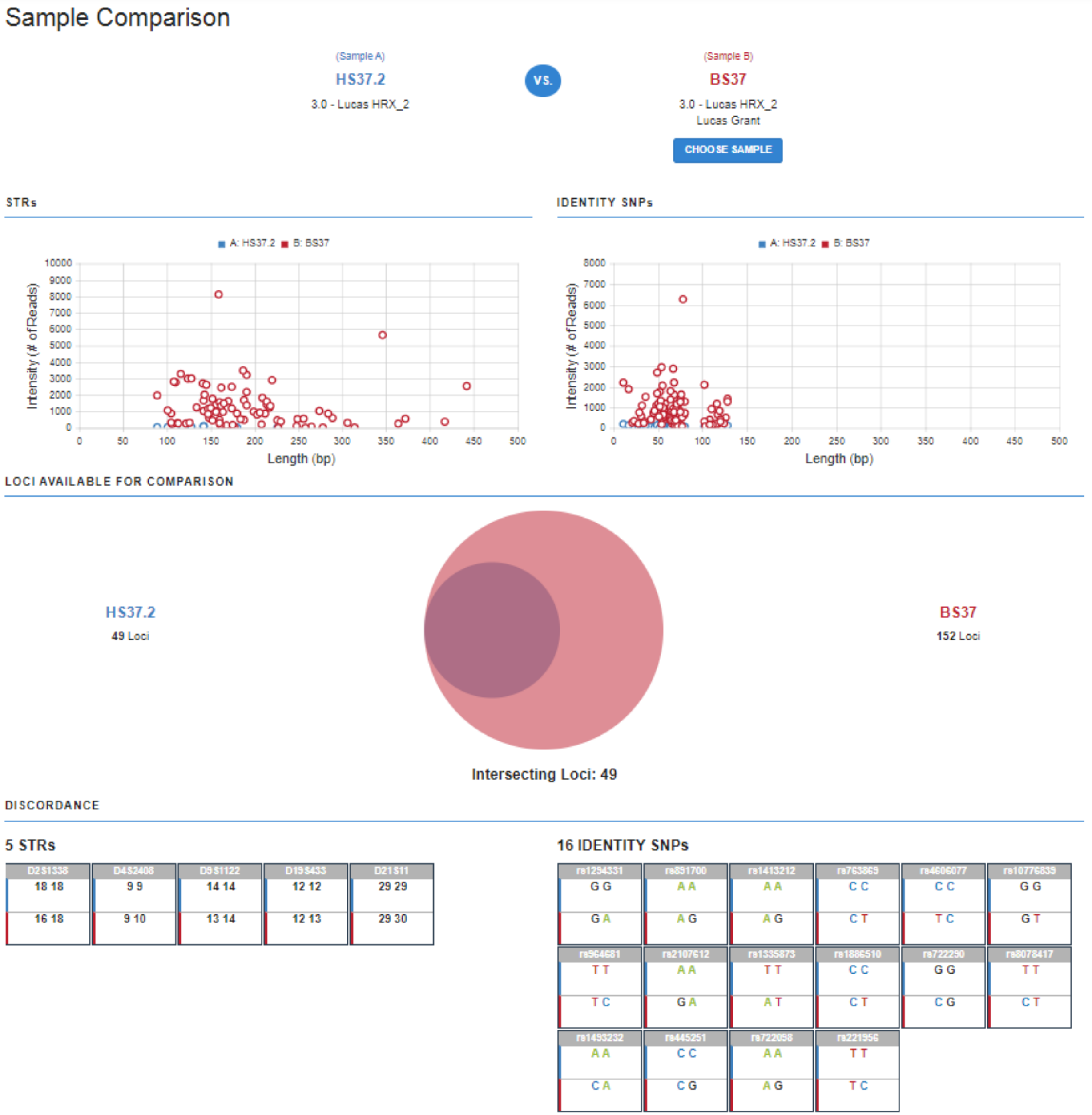
DNA Signature Prep Hair Data

- 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

(Sample B)
BS37
3.0 - Lucas HRX_2
Lucas Grant

Intersecting Loci: 49

DISCORDANCE

5 STRs

D2S1338	D4S2408	D9S1122	D19S433	D21S11
18 18	9 9	14 14	12 12	29 29
16 18	9 10	13 14	12 13	29 30

16 IDENTITY SNPs

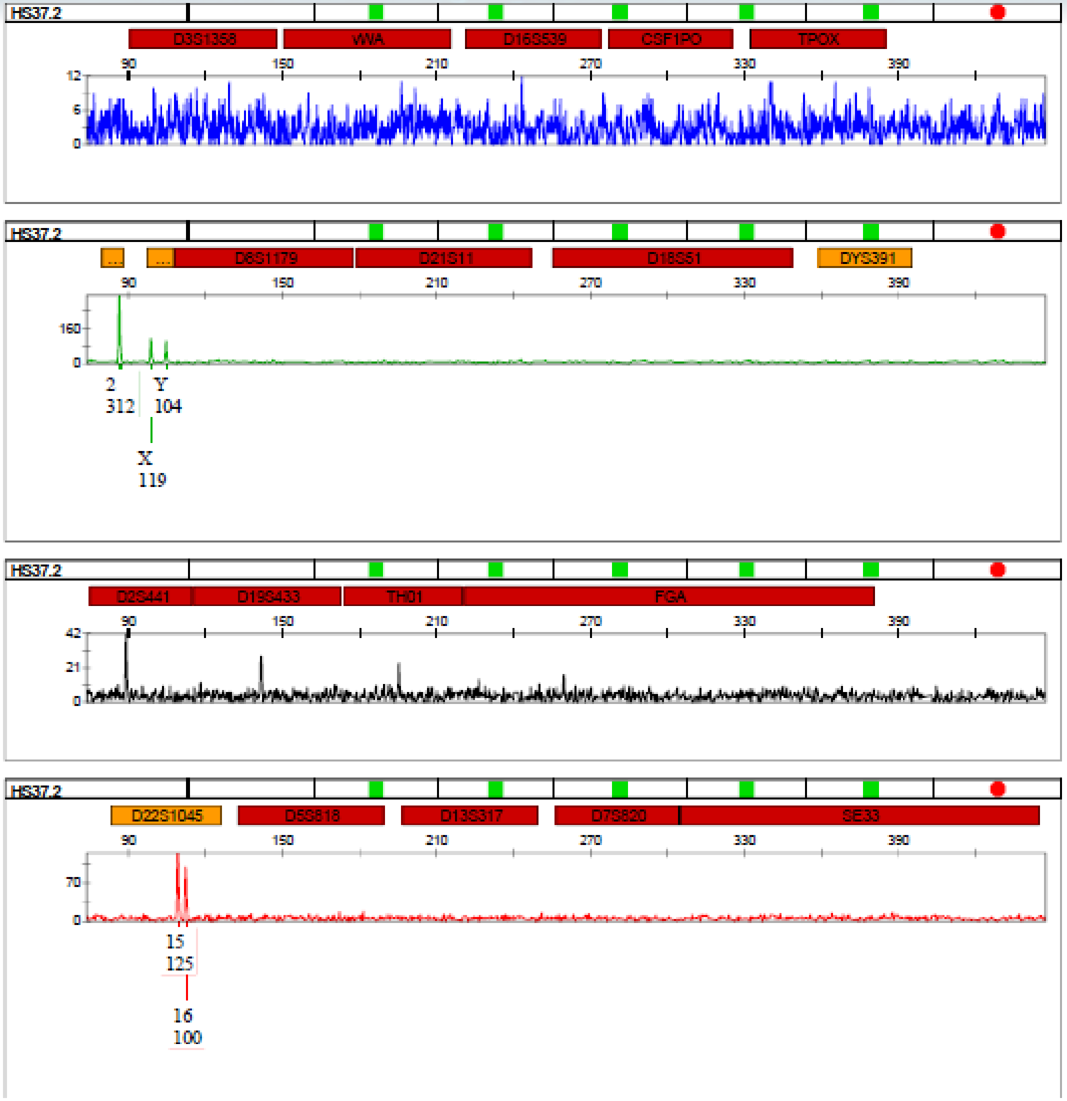
rs1294331	rs891700	rs1413212	rs763869	rs4606077	rs10776839
G G	A A	A A	C C	C C	G G
G A	A G	A G	C T	T C	G T

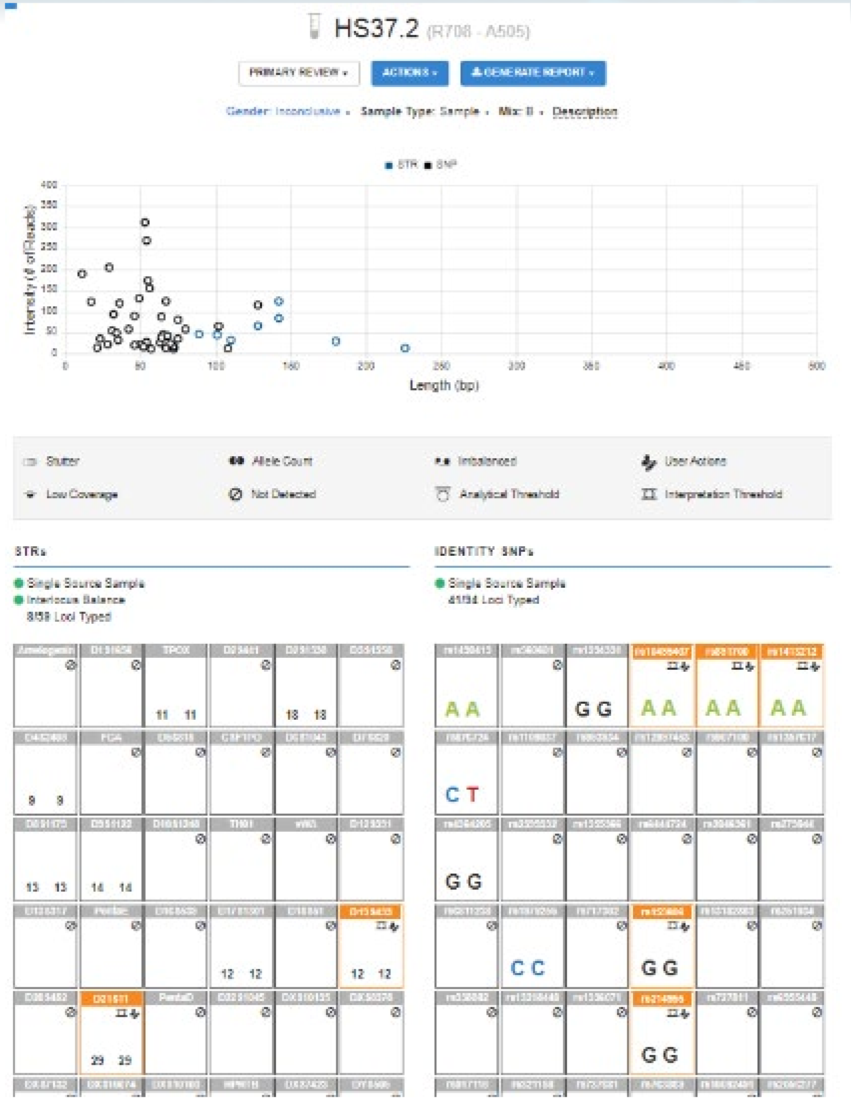
rs964681	rs2107612	rs1335873	rs1886510	rs722290	rs8078417
T T	A A	T T	C C	G G	T T
T C	G A	A T	C T	C G	C T

rs1493232	rs445251	rs722098	rs221956
A A	C C	A A	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




HS37.2 (R708 - A505)

Gender: Inconclusive ▾ Sample Type: Sample ▾ Mix: ll ▾ Description

STRs

● Single Source Sample
● Interlocus Balance
8/58 Loci Typed

IDENTITY SNPs

● Single Source Sample
41/94 Loci Typed

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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FSF Emerging Forensic Scientist Award
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Questions?

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