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The Development of a Five-Dye Insertion/Deletion (INDEL) Panel for Human Identification

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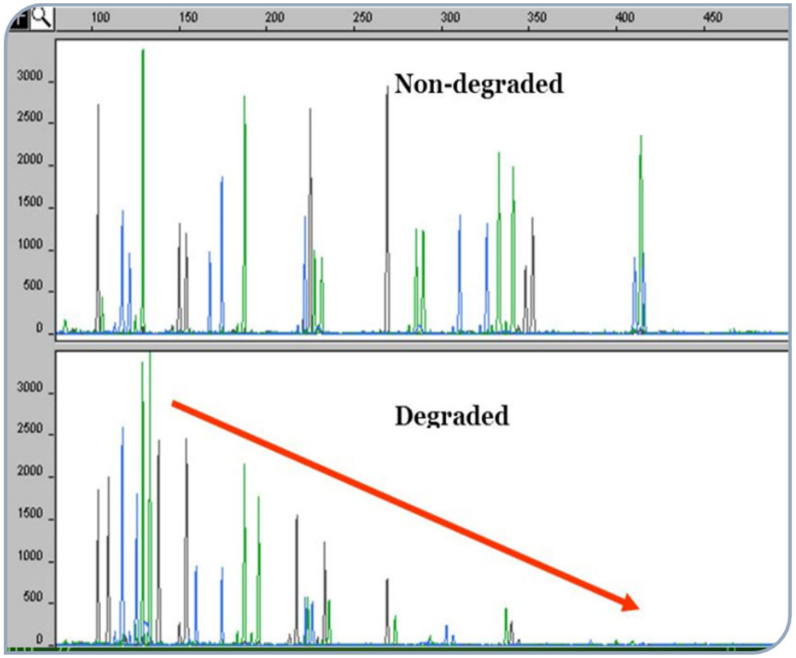
Disclaimer

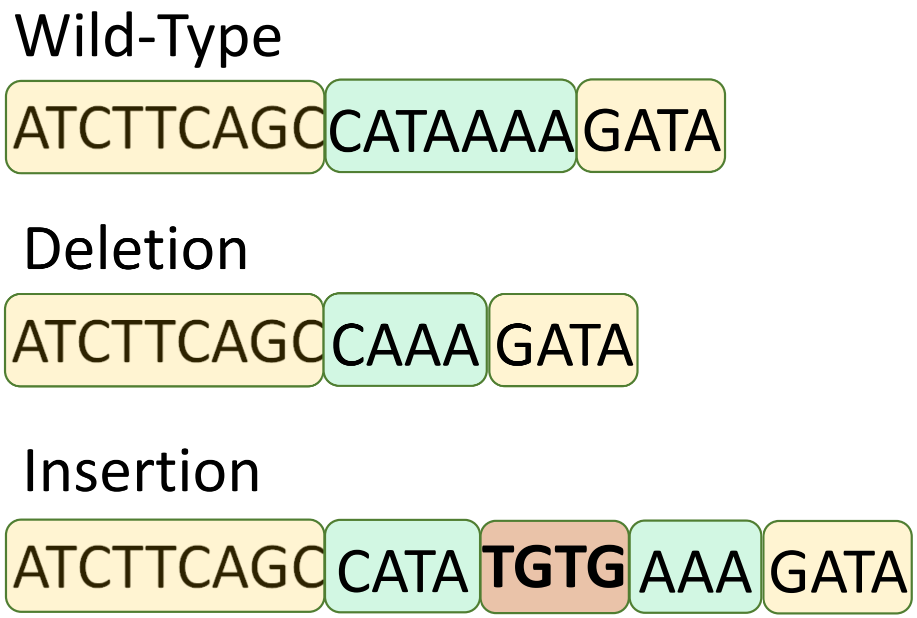
There are no conflicts of interest related to the content of this presentation. The opinions, findings, conclusions, or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of Sam Houston State University or any other.

Challenging Samples and Limited recovery



- Twenty core Short Tandem Repeats(STR) CODIS loci are the standard
- There are limitations
- DNA degradation results in a ski slope
  - Environment
  - Aged
- Alternative markers can provide options if recovery is low
  - These include SNPs and INDELs
  - Effectively use smaller amplicons

Insertion Deletion Polymorphisms



Wild-Type

ATCTTCAGC CATAAAA GATA

Deletion

ATCTTCAGC CAAA GATA

Insertion

ATCTTCAGC CATA **TGTG** AAA GATA

- Contains a **long** and **short** allele
- Can range from 1 to hundreds of nucleotides
- Small amplicon design (<200 bp) possible
- Success with degraded samples

Kit Options

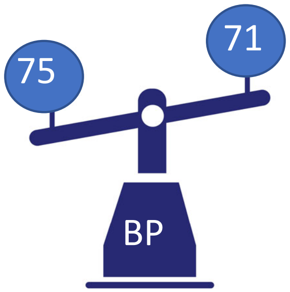
- Lower *Fst*
- High mutations
- Effective distinguishing markers
- HID-INDEL **focus** of the presentation
- Vary from individual to individual

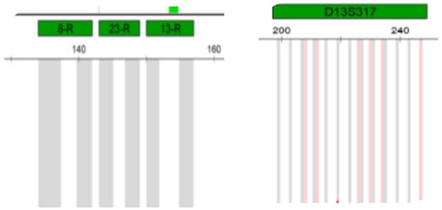
- Higher *Fst* compared to traditional HID markers
- AIM-INDELs kit previously tested
  - Vary in different populations due to fixation

Goals

Effectively	Effectively differentiate HID markers within the kit and between samples
Validate	Validate the kit for use with a variety of sample types
Limitations	Determine limitations of the kit with low quantity or challenging samples
Discussion	Discuss conclusions and review fusion of the AIMS and HID markers

Considerations Moving Forward





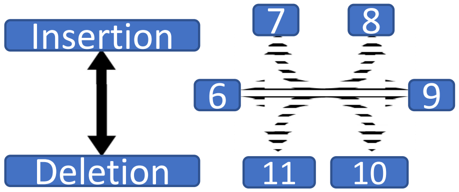
INDEL application mimics STR methods

(size based comparison)

Discriminatory Power per locus

Size Selection / loci per base

No slippage = ease of analysis



Overview

Sample Collection

Buccal swabs (n= 85 Samples)
DNA Extraction
DNA Quantification

Multiplex optimization

Marker selection
Optimize PCR conditions
Electrophoresis Analysis

Validation

Sensitivity
Mixtures
Challenging Samples
Principal Component Analysis

Conclusions

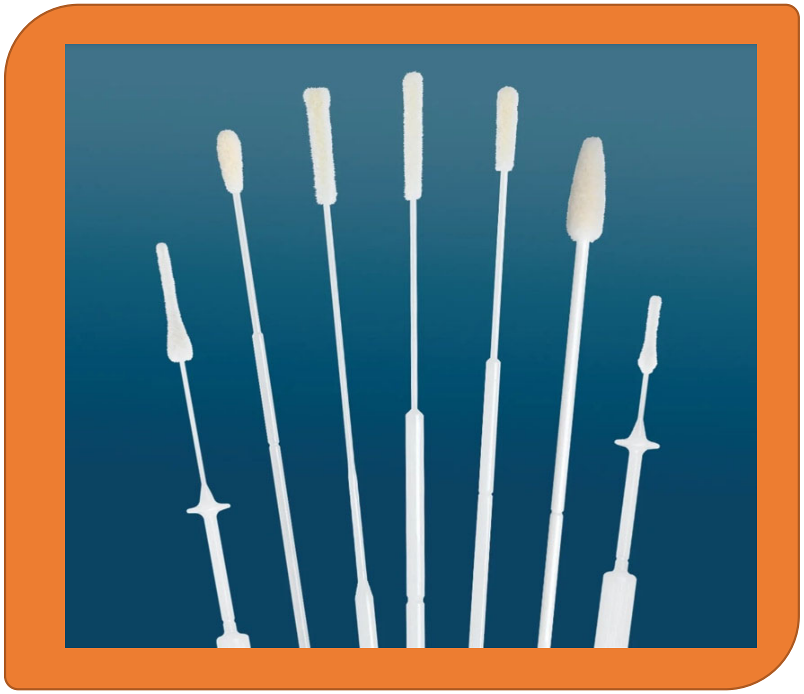
Review
Considerations
Thoughts

Future work

Ancestry kit
Uninformative markers
INDEL kit comparisons

Sample Acquisition

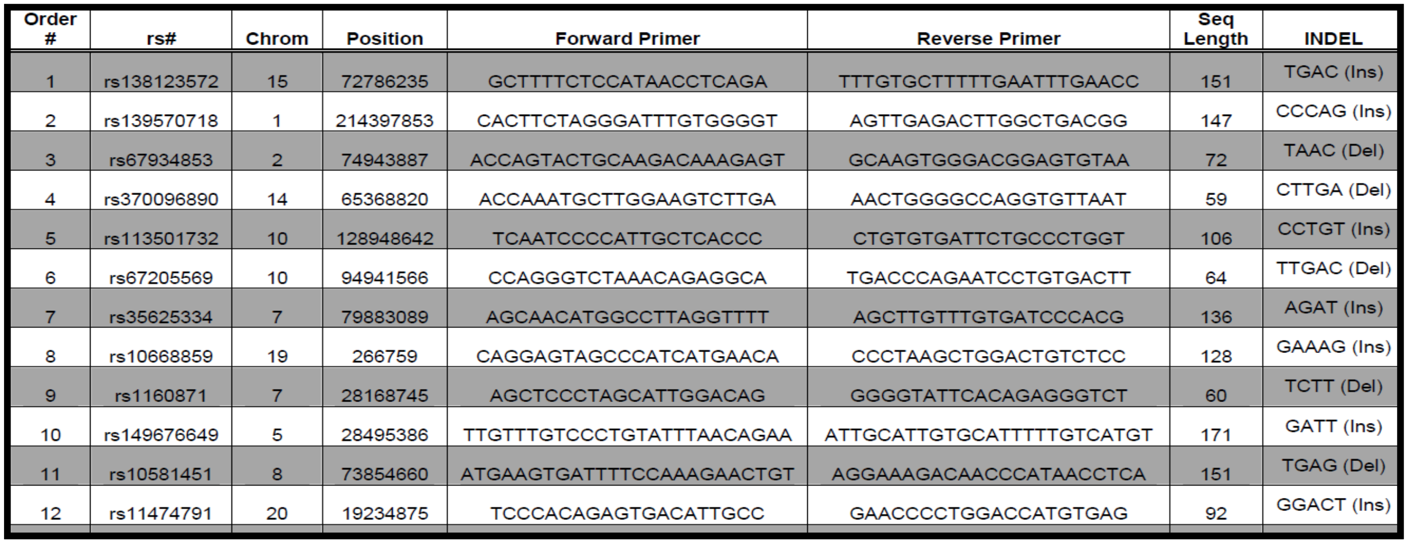
- Buccal swabs were collected according to approved protocols of the institutional review board for the protection of human subjects at Sam Houston State University
- Buccal were obtained from:
  - 37 Caucasian
  - 15 East Asian
  - 11 African American
  - 19 Hispanic
  - 3 Other



Marker selection

- 1000 genome project data
- 33 markers across 5 dye channels
- Amplicon size: 50 to 215bp
- low Fst

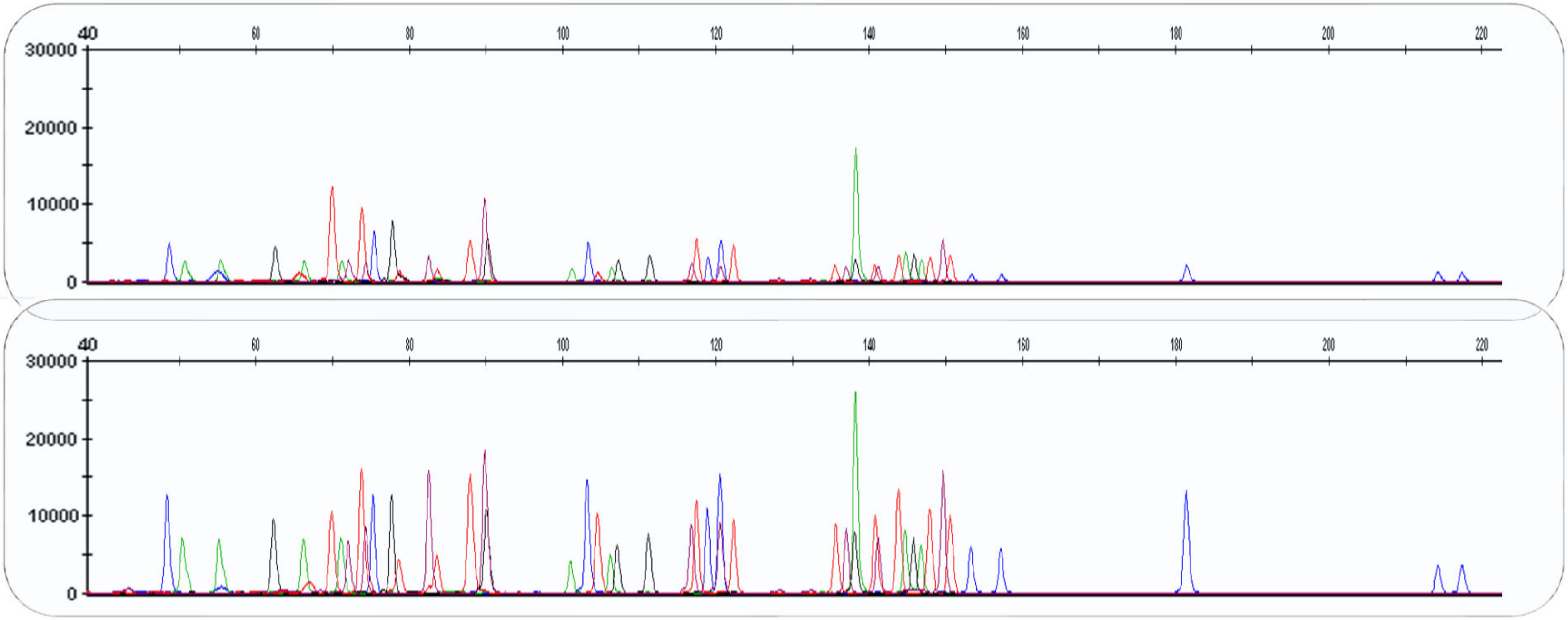
- Separated and Detected on 3500 Genetic analyzer
- Analyzed data with Genemapper ID-X software



Order #	rs#	Chrom	Position	Forward Primer	Reverse Primer	Seq Length	INDEL
1	rs138123572	15	72786235	GCTTTTCTCCATAACCTCAGA	TTTGTGCTTTTTGAATTTGAACC	151	TGAC (Ins)
2	rs139570718	1	214397853	CACTTCTAGGGATTTGTGGGGT	AGTTGAGACTTGGCTGACGG	147	CCCAG (Ins)
3	rs67934853	2	74943887	ACCAGTACTGCAAGACAAAGAGT	GCAAGTGGGACGGAGTGTAA	72	TAAC (Del)
4	rs370096890	14	65368820	ACCAAATGCTTGGAAGTCTTGA	AACTGGGGCCAGGTGTTAAT	59	CTTGA (Del)
5	rs113501732	10	128948642	TCAATCCCCATTGCTCACCC	CTGTGTGATTCTGCCCTGGT	106	CCTGT (Ins)
6	rs67205569	10	94941566	CCAGGGTCTAAACAGAGGCA	TGACCCAGAATCCTGTGACTT	64	TTGAC (Del)
7	rs35625334	7	79883089	AGCAACATGGCCTTAGGTTTT	AGCTTGTTTGTGATCCCACG	136	AGAT (Ins)
8	rs10668859	19	266759	CAGGAGTAGCCCATCATGAACA	CCCTAAGCTGGACTGTCTCC	128	GAAAG (Ins)
9	rs1160871	7	28168745	AGCTCCCTAGCATTGGACAG	GGGGTATTCACAGAGGGTCT	60	TCTT (Del)
10	rs149676649	5	28495386	TTGTTTGTCCCTGTATTTAACAGAA	ATTGCATTGTGCATTTTTGTCATGT	171	GATT (Ins)
11	rs10581451	8	73854660	ATGAAGTGATTTTCCAAAGAACTGT	AGGAAAGACAACCCATAACCTCA	151	TGAG (Del)
12	rs11474791	20	19234875	TCCCACAGAGTGACATTGCC	GAACCCCTGGACCATGTGAG	92	GGACT (Ins)

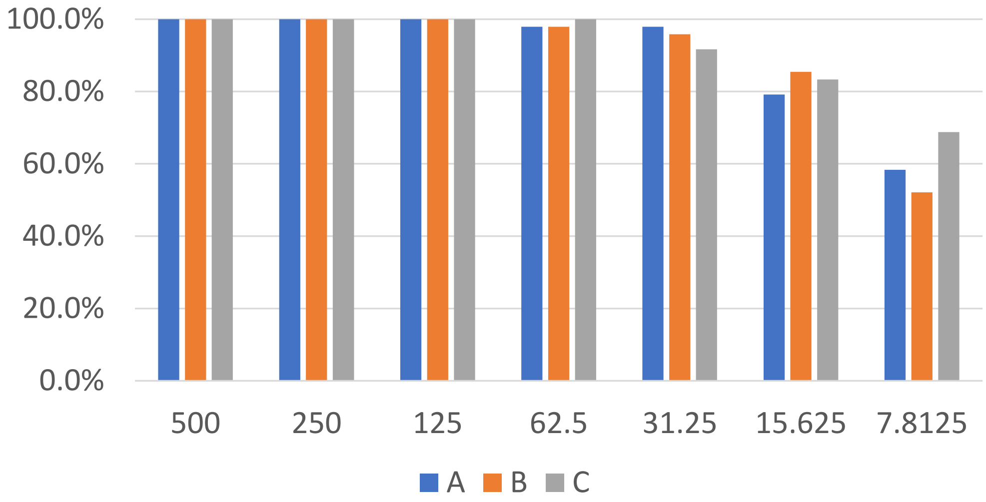
Primer Balance

- Final primer titrations using the 3500 Genetic Analyzer
- Final multiplex with 33 primer pairs using 007 controlled DNA and buccal swabs was successful
- Optimized and balanced using 500pg

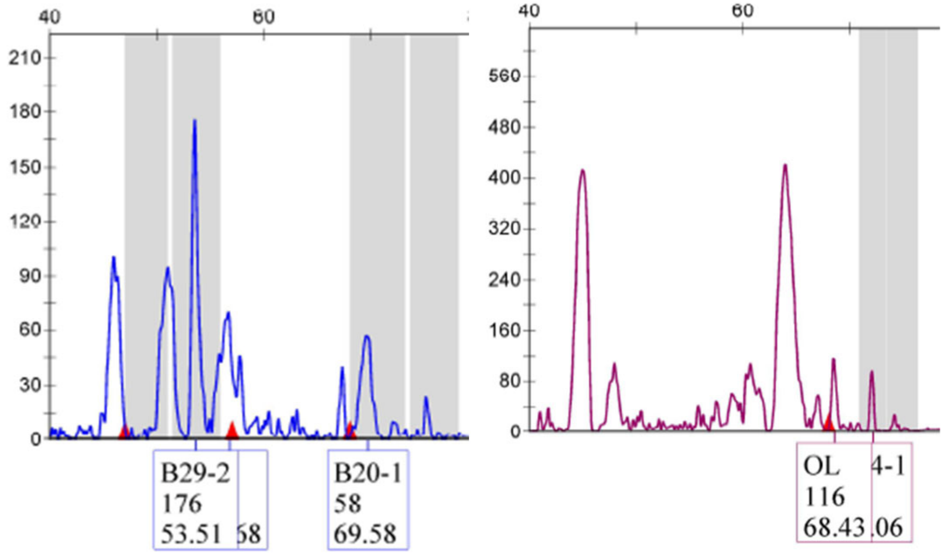


Sensitivity

- A serial dilution in triplicate performed with an input range from 500pg-8pg:
  - Multiplex is sensitive with allele dropout beginning at 62.5pg
  - 8pg input recover between 52-69% of expected alleles

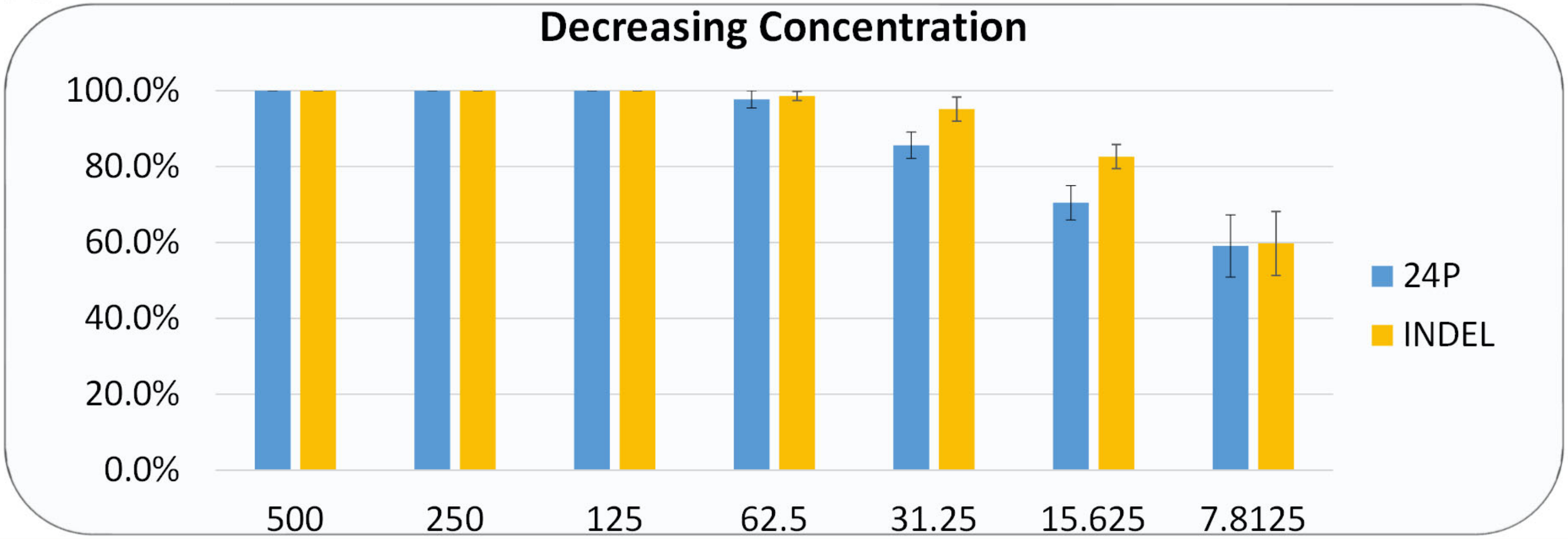


- Analysis at low input
  - Low input samples may have primer dimer or noise
  - Interpretation at small amplicons may be complex



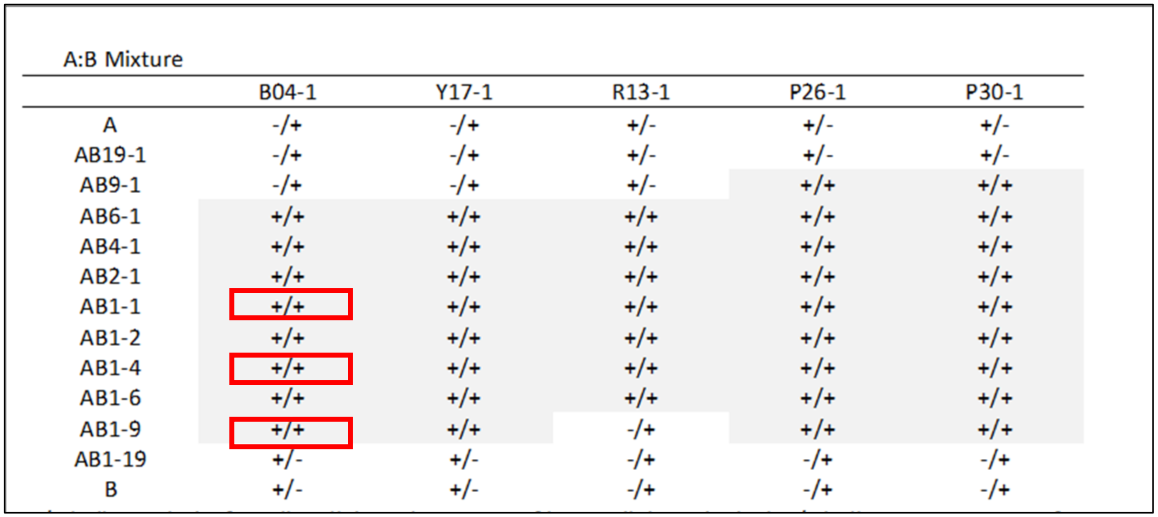
Sensitivity Comparison

- The serial dilution in triplicate:
  Investigator 24 plex and INDEL kit with input range from 500pg-8pg:
  - Similar range observed
  - Average recovery shown
  - Drop out of expected alleles at 62.5pg
  - At 8pg recovery is between 50-66%



Mixture Analysis

- Assessed Mixtures of A:B, A:C, B:C in ratios:
  - Ratios up to 1:9 could recover the minor donor when considering non-shared alleles
  - Out of the triplicate some minor alleles were not observed at 1:6 and 1:9



A:B Mixture

	B04-1	Y17-1	R13-1	P26-1	P30-1
A	-/+	-/+	+/-	+/-	+/-
AB19-1	-/+	-/+	+/-	+/-	+/-
AB9-1	-/+	-/+	+/-	+/+	+/+
AB6-1	+/+	+/+	+/+	+/+	+/+
AB4-1	+/+	+/+	+/+	+/+	+/+
AB2-1	+/+	+/+	+/+	+/+	+/+
AB1-1	+/+	+/+	+/+	+/+	+/+
AB1-2	+/+	+/+	+/+	+/+	+/+
AB1-4	+/+	+/+	+/+	+/+	+/+
AB1-6	+/+	+/+	+/+	+/+	+/+
AB1-9	+/+	+/+	-/+	+/+	+/+
AB1-19	+/-	+/-	-/+	-/+	-/+
B	+/-	+/-	-/+	-/+	-/+

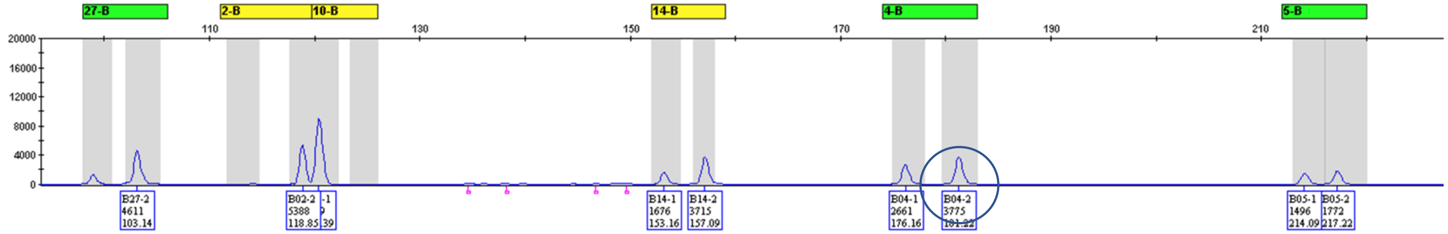
Homozygous to Heterozygous

Shared Alleles

Heterozygous to Homozygous

Non-shared

Shared Alleles



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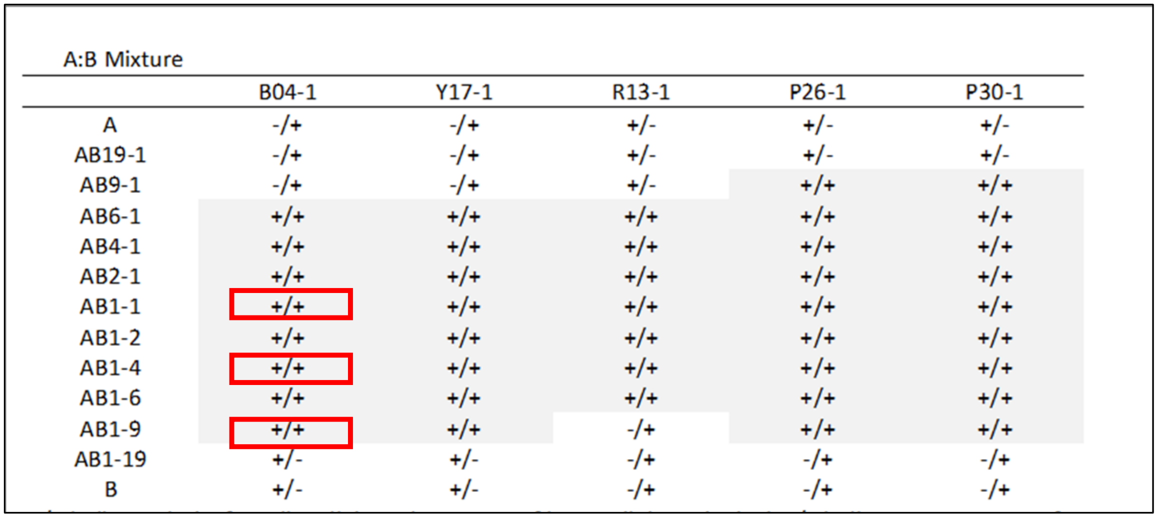

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AB1-1	+/+	+/+	+/+	+/+	+/+
AB1-2	+/+	+/+	+/+	+/+	+/+
AB1-4	+/+	+/+	+/+	+/+	+/+
AB1-6	+/+	+/+	+/+	+/+	+/+
AB1-9	+/+	+/+	-/+	+/+	+/+
AB1-19	+/-	+/-	-/+	-/+	-/+
B	+/-	+/-	-/+	-/+	-/+

Homozygous to Heterozygous

Shared Alleles

Heterozygous to Homozygous

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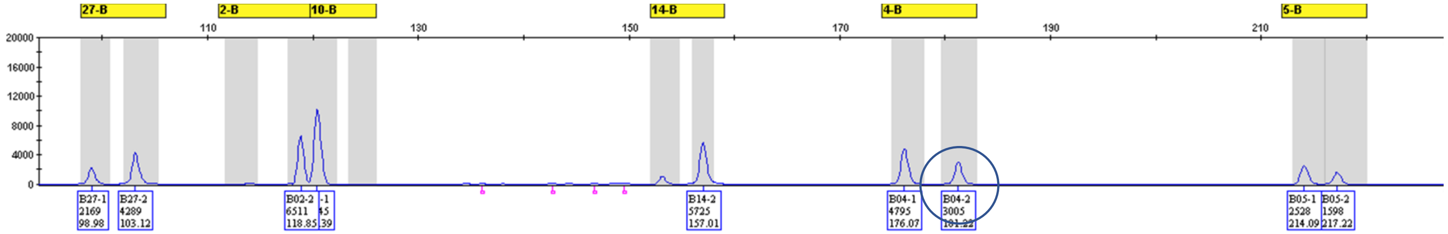
Shared Alleles



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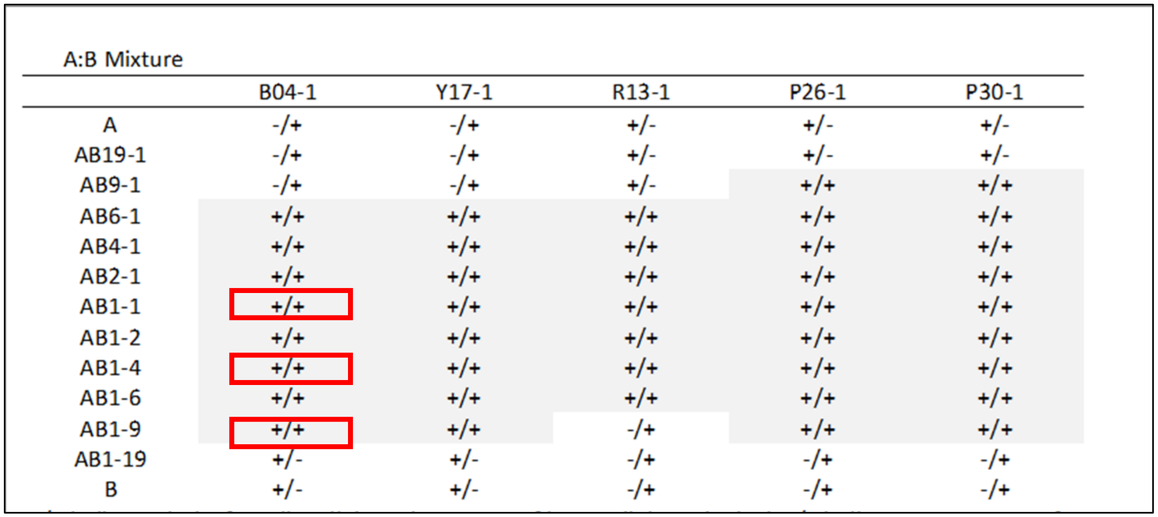

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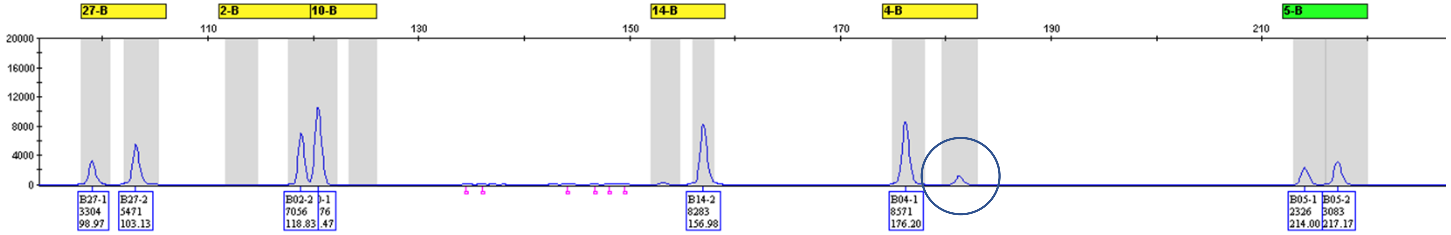
AB1-1	+/+	+/+	+/+	+/+	+/+
AB1-2	+/+	+/+	+/+	+/+	+/+
AB1-4	+/+	+/+	+/+	+/+	+/+
AB1-6	+/+	+/+	+/+	+/+	+/+
AB1-9	+/+	+/+	-/+	+/+	+/+
AB1-19	+/-	+/-	-/+	-/+	-/+
B	+/-	+/-	-/+	-/+	-/+



Homozygous to Heterozygous

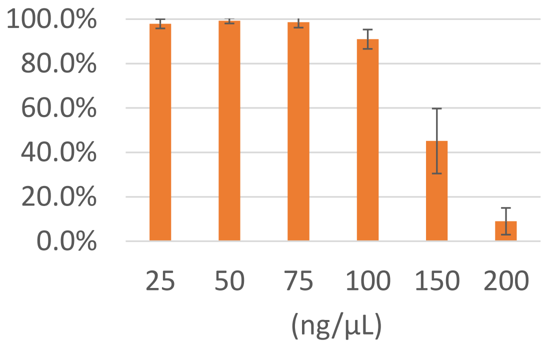
Shared Alleles

Heterozygous to Homozygous

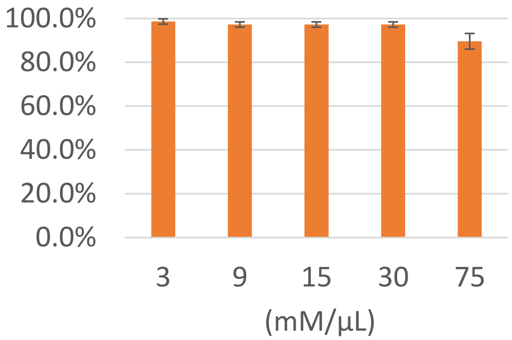
Non-shared

Shared Alleles

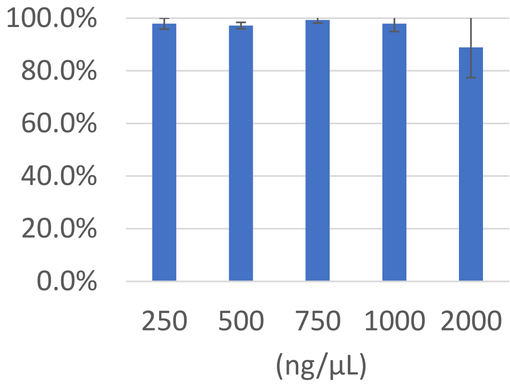
Inhibition



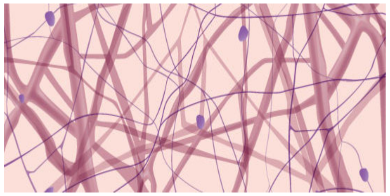
Humic Acid

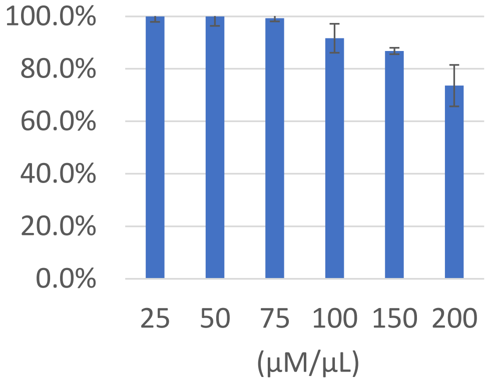


Calcium

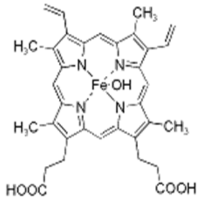


Collagen

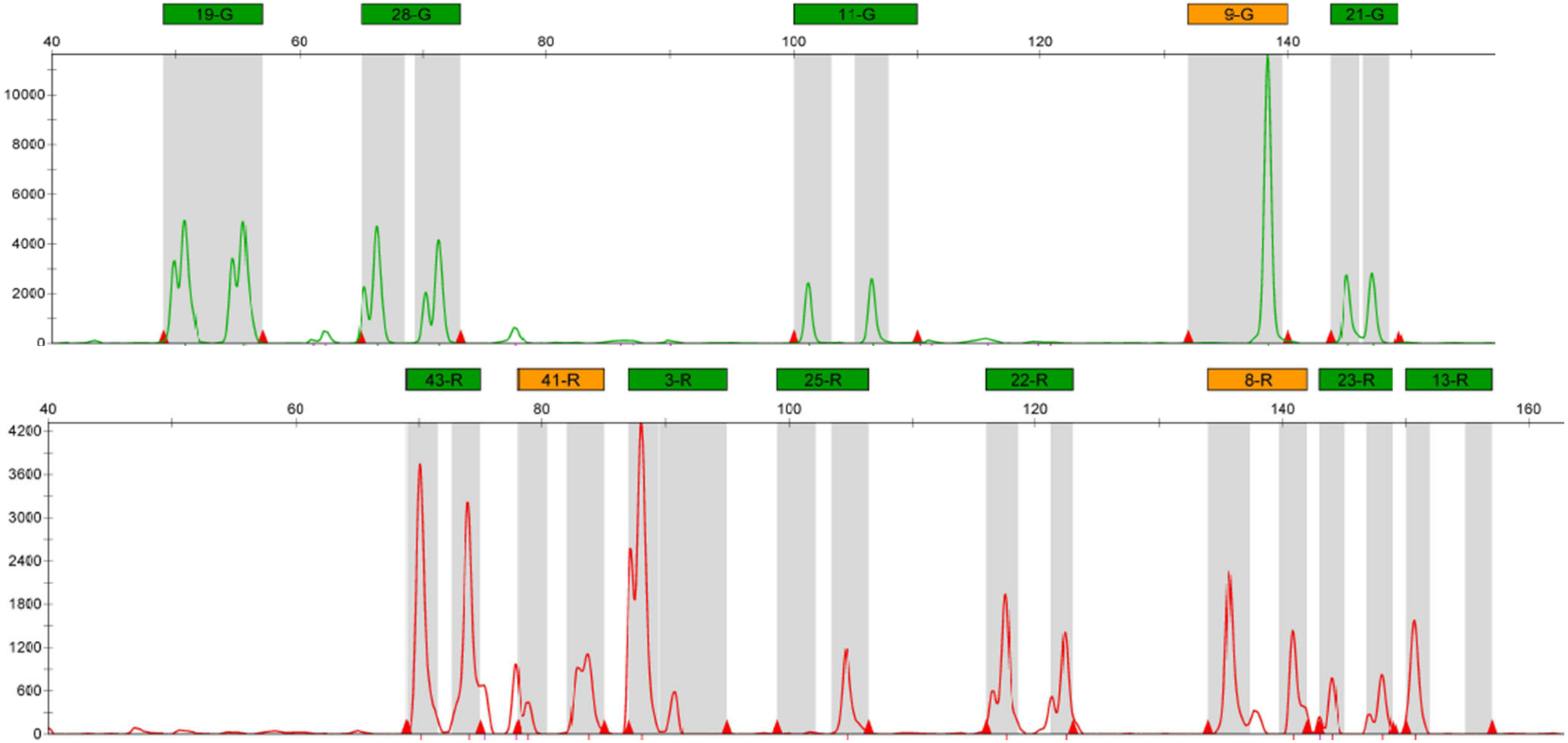




Hematin

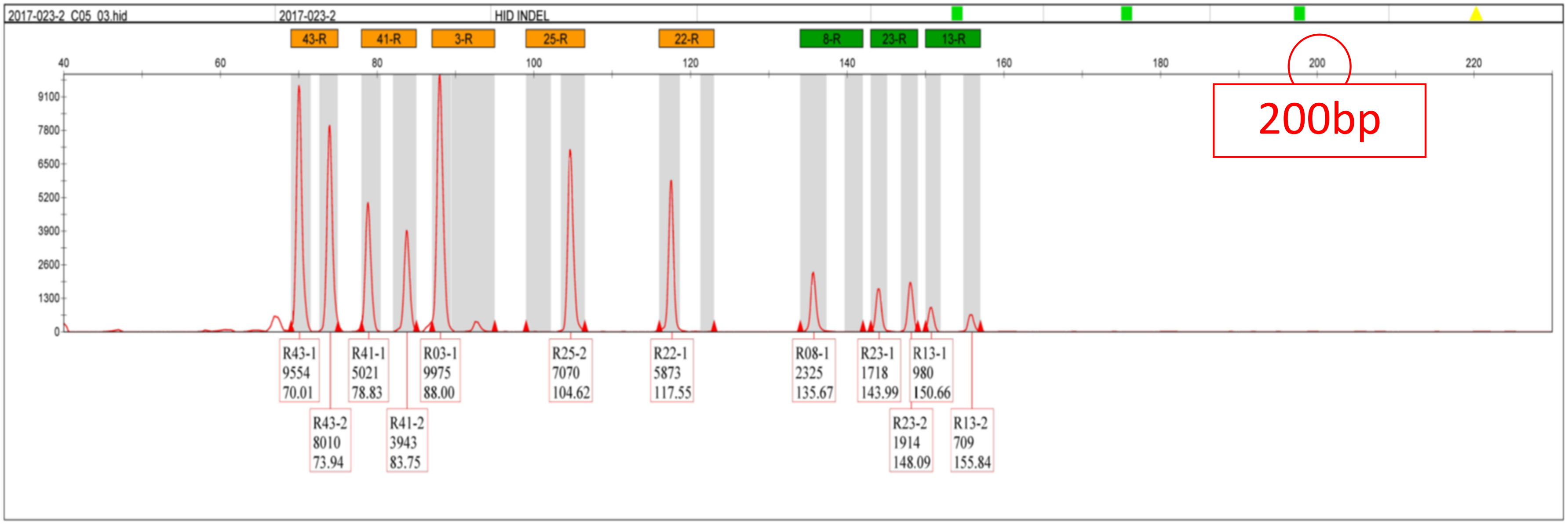


Inhibition


19-G
28-G
11-G
9-G
21-G
40
60
80
100
120
140
10000
8000
6000
4000
2000
0
43-R
41-R
3-R
25-R
22-R
8-R
23-R
13-R
40
60
80
100
120
140
160
4200
3600
3000
2400
1800
1200
600
0

Challenging Samples

- Bone and Hair samples were ran using the assay and compared to a commercial kit
- Short amplicons should allow for improved performance with degraded samples



200bp

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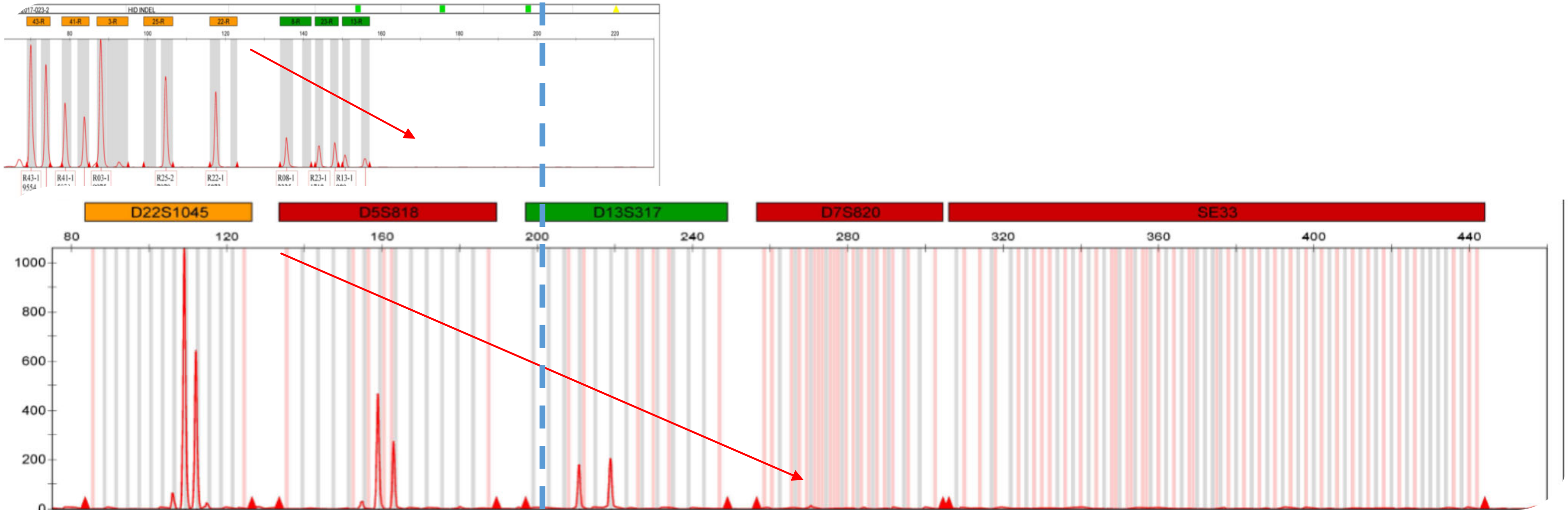
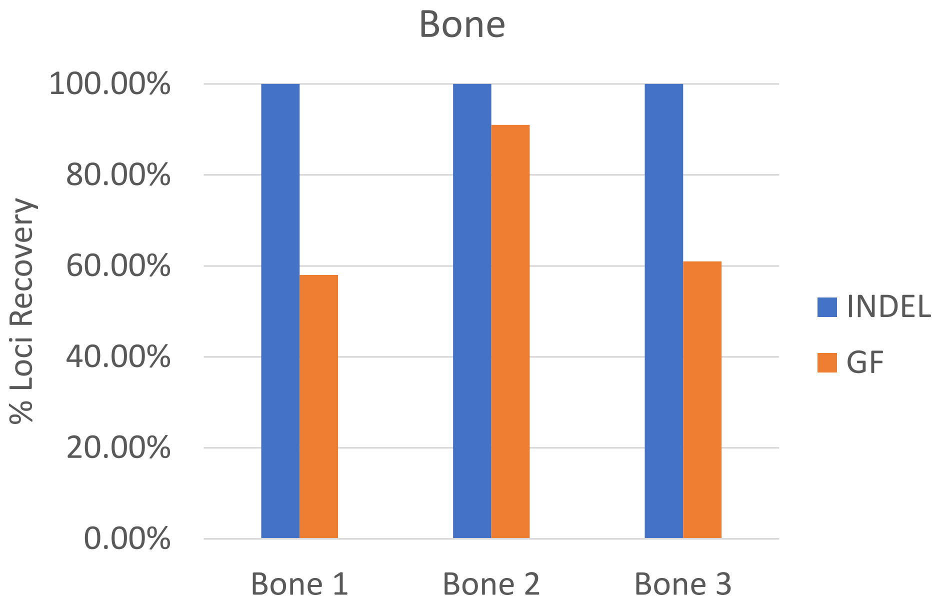
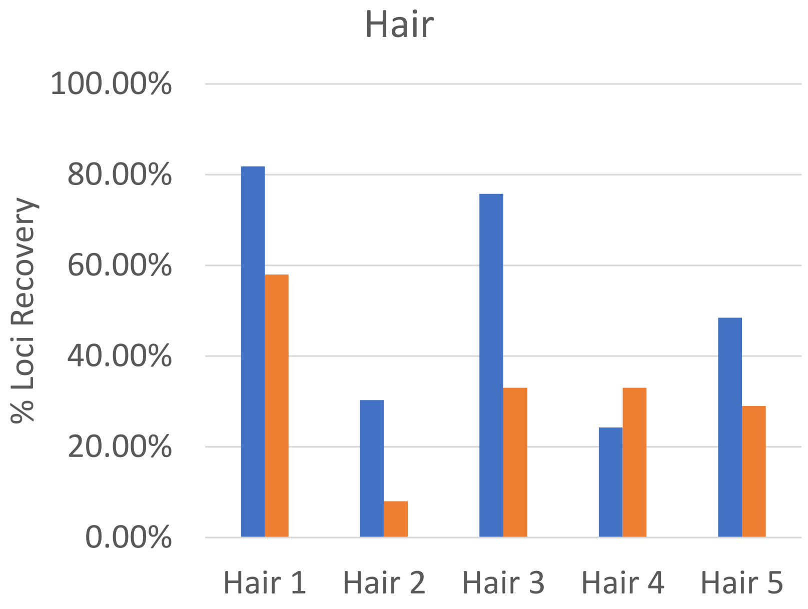


200bp

Challenging Comparison



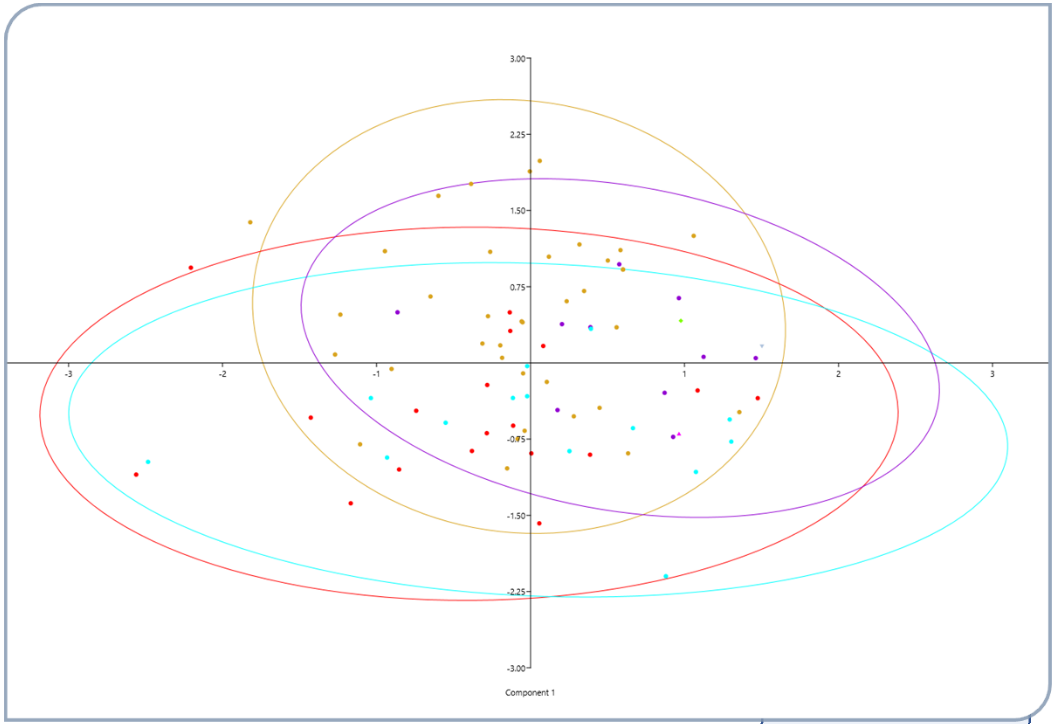


Bone samples input 500pg for each kit

Hair samples were low quantity and could not input 500pg
GlobalFiler™ maximum input DNA (15 µL) vs. INDEL (~ 5 µL)

Population Overview

- Population separation not observed using PCA plots (PAST4)
- Population groups labeled as:
  - Caucasian-Gold
  - African American-Purple
  - East Asian- Light Blue
  - Hispanic-Red
- Single sample analysis:
  - 3 other admixed



95% ellipses

Conclusion

- ✓ The INDEL kit was able to obtain full profiles to 62pg and partial recovery at 8pg between 50-66%
- Challenging samples were comparable with GlobalFiler™ at lower amounts of input DNA
- Preliminary results indicate that selected HID markers can distinguish individuals with the population size currently
- Can easily be used in conjunction with current investigative methods for casework type samples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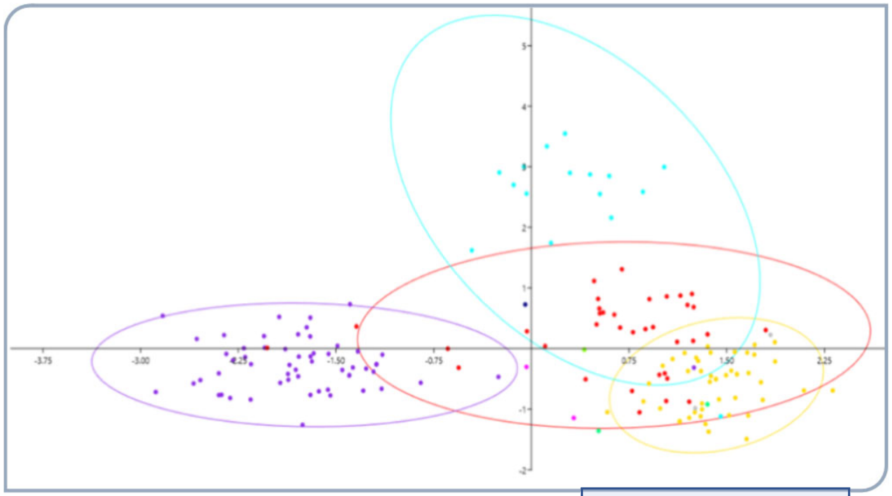
ONE
MORE
THING

Future work: Hybrid Panel Marker Selection

Previous Ancestry Panel:

Population groups labeled as:

- Caucasian-Gold
- African American-Purple
- East Asian- Light Blue
- Hispanic-Red



95% ellipses

Marker Review within population study,
In progress

Allele	INDEL-AIM Y6 ☑	INDEL-AIM P20 ☒
Pop	A	A
% het	5%	**47%**
% hom 1	2%	**33%**
% hom 2	**93%**	**19%**

Expect grouping

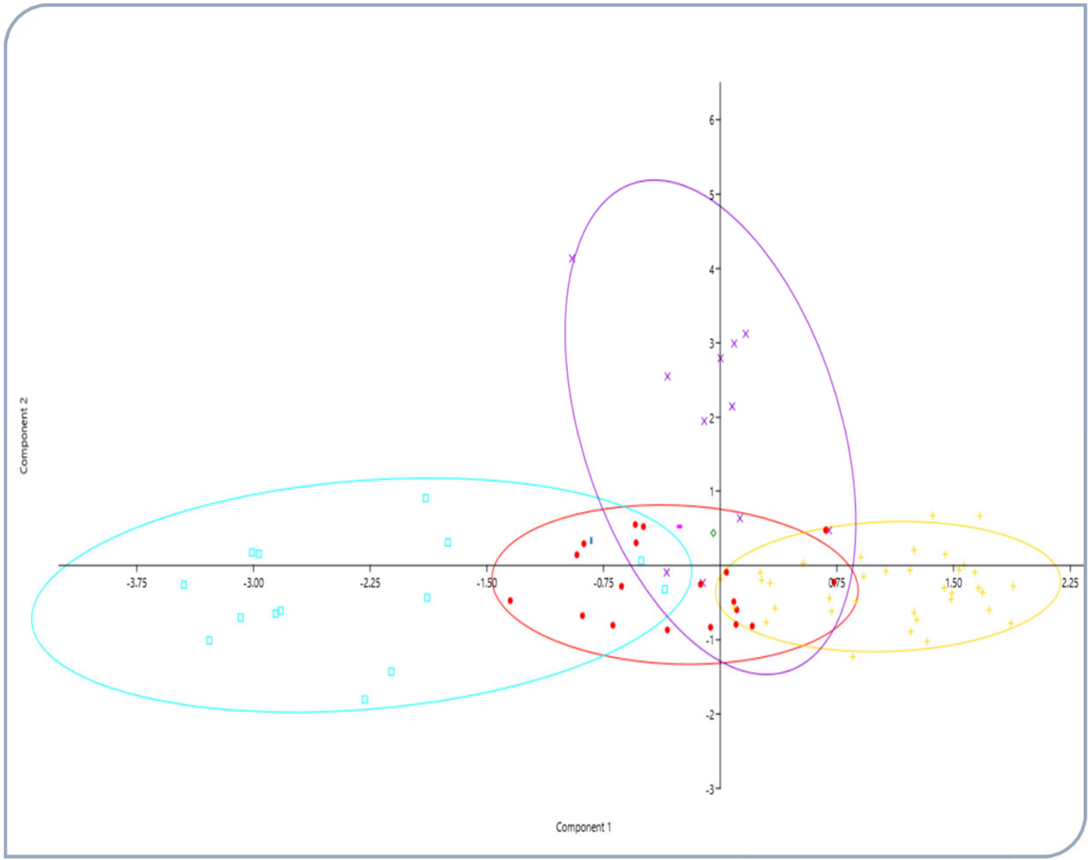
Allele	INDEL-HID R22 ☑	INDEL-HID Y01 ☒
% het	**46.6%**	4.5%
% hom 1	**30.7%**	**94.3%**
% hom 2	**20.5%**	0.0%

Expect variance

Future work: Hybrid Panel Testing

PCA shown using data from both kits

- All markers from each study combined
- N=81 samples used in both studies
- Goals:
  - One kit for all
  - Determine effectiveness
  - Reduce Loss of unique identification
  - Reduce Loss of grouping populations power
  - Continued effectiveness with low sensitivity and challenging sample recovery
- Marker selection needs to be completed
- More population samples



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Questions?

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