Streamlining Degraded Sample Processing in Forensic Laboratories: Developing a Decision Tree for Capillary Electrophoresis and Next Generation Sequencing Platforms

Rachel Houston, PhD; Natalia Czado, MS; Damani Johnson, BS;
Lucio Avellaneda, BS; Cesar Cantu, BS; Sheree Hughes; PhD

Department of Forensic Science

Sam Houston State University

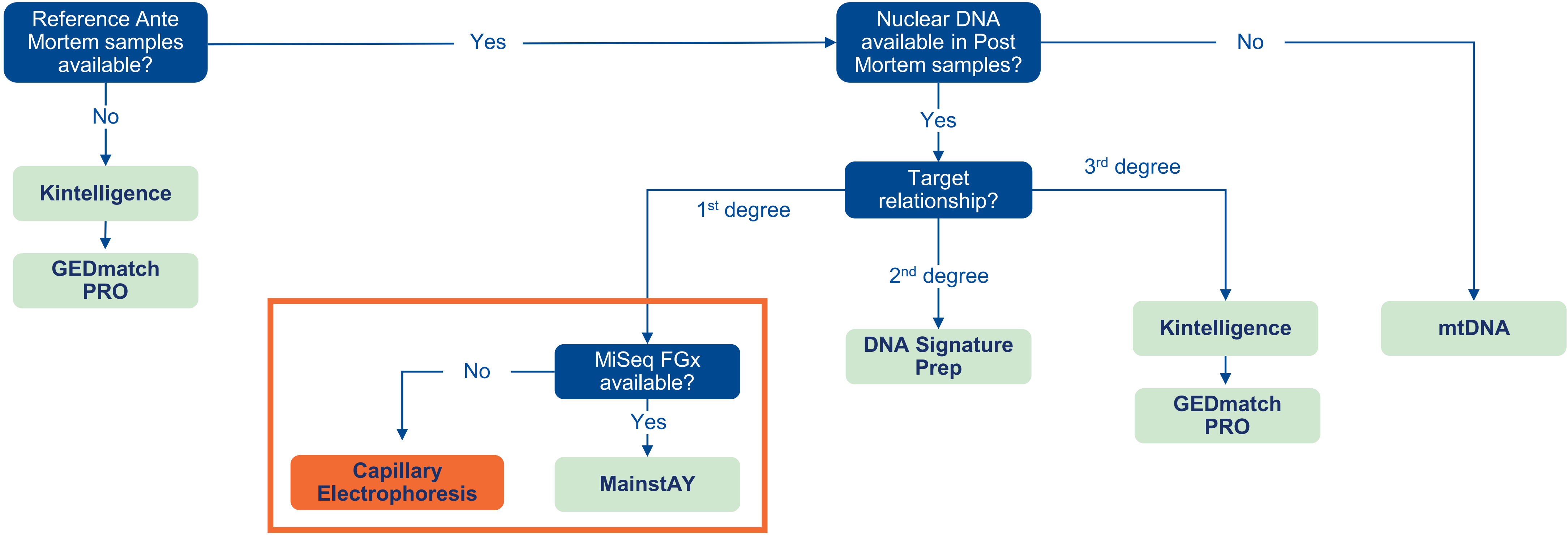
Disclaimer

- The views and opinions expressed in this presentation are those of the presenter and does not represent any official views or opinions of the American Academy of Forensic Sciences.

Disclaimer

- QIAGEN is not affiliated with the Department of Forensic Science at Sam Houston State University in the United States. The views expressed herein are those of the speakers, and do not necessarily express the views of QIAGEN.
- SHSU was provided instrumentation, consumables, and travel support by QIAGEN, but no remuneration.
- Due to the sensitive material, photographs of human remains are **NOT** permitted

Unidentified Remains Decision Tree



Reference Ante Mortem samples available?

Yes

No

Kintelligence

GEDmatch PRO

Nuclear DNA available in Post Mortem samples?

No

mtDNA

Yes

Target relationship?

1st degree

MiSeq FGx available?

No

Capillary Electrophoresis

Yes

MainstAY

2nd degree

DNA Signature Prep

3rd degree

Kintelligence

GEDmatch PRO

Research Questions

1. What if your laboratory has both a MiSeq and a CE?
2. Are there any benefits to using NGS STR chemistry over a CE STR chemistry?

FBI approves QIAGEN's NGS-based ForenSeq MainstAY workflow for the U.S. National DNA Index System (NDIS)

June 20, 2023

- Degradation
  - The ForenSeq™ MainstAY Kit contains 21 autosomal STR loci completely less than 250bp, whereas the Investigator® 24plex QS Kit has only 10 autosomal STR loci completely less than 250bp
- Statistical weighting
  - Isoallele frequencies and increased number of STRs

Degraded Samples

- Artificially degraded samples
  - DNase treatment
  - Not as realistic as environmentally degraded samples

- Burnt skeletal remains

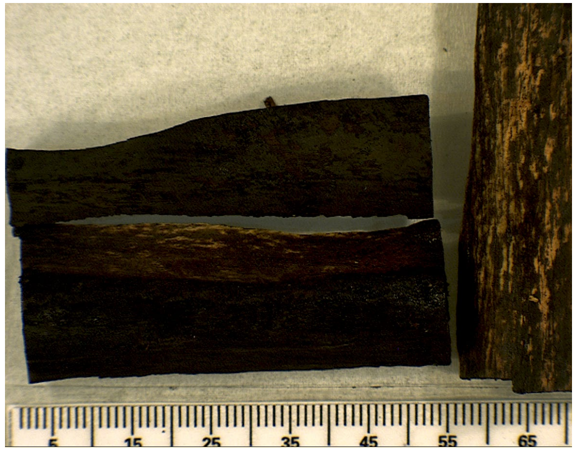
SOUTHEAST TEXAS APPLIED FORENSIC SCIENCE FACILITY

Femur Fragments Thermally Degraded at Different Levels

Unburned

Burned, Light Brown

Burned, Dark Brown



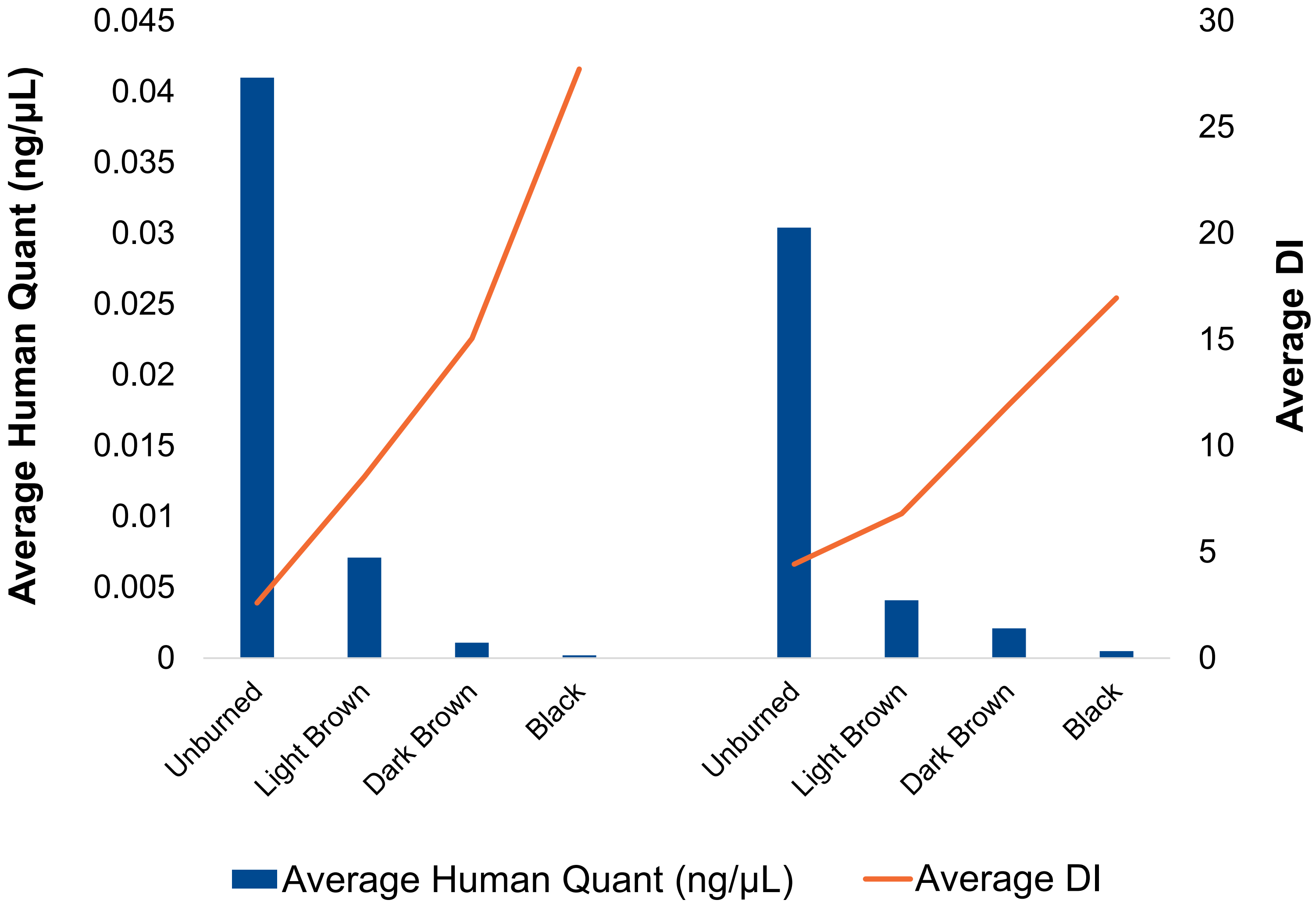
Burned, Black

- 2 cadavers, 5 replicates at each thermal degradation level

DNA Extraction and Quantification

- All bones (**250 mg**) were extracted using the extra-large volume protocol on the EZ2 Connect Fx and eluted in **100 µL**
- All extracts were quantified using Investigator Quantiplex Pro

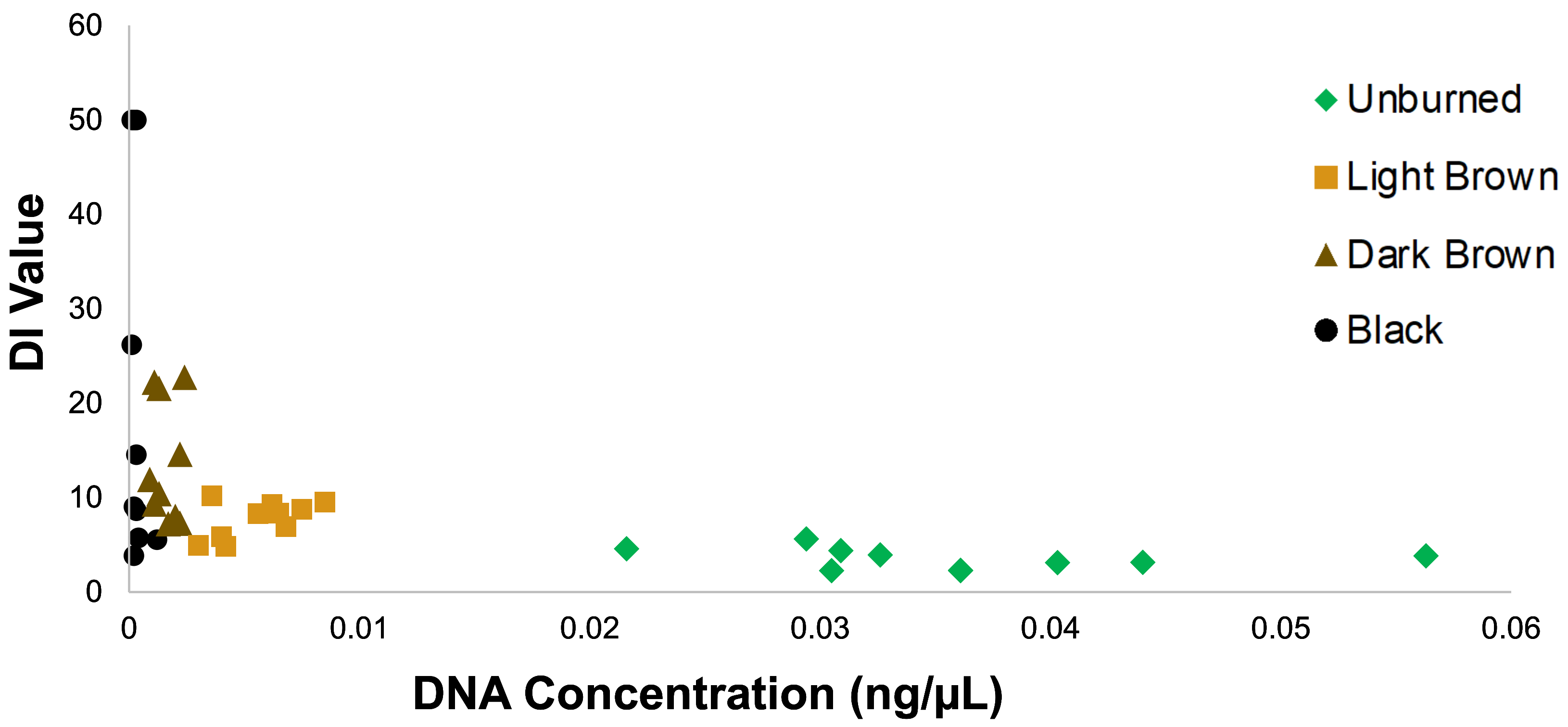
Average DNA Concentration vs. Average Degradation



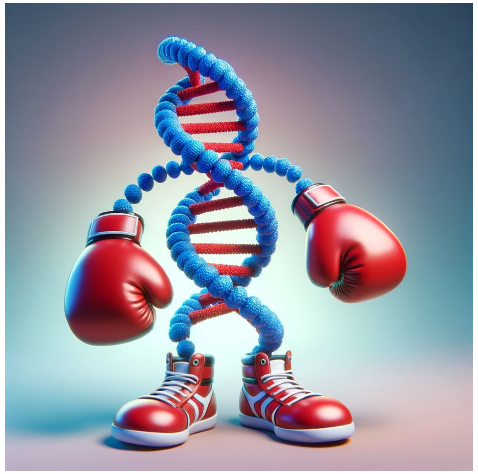
N=5 replicates

- Both cadavers displayed the same pattern of increasing DI values and decreasing DNA concentration with more thermal degradation

Degradation Index vs. DNA Concentration



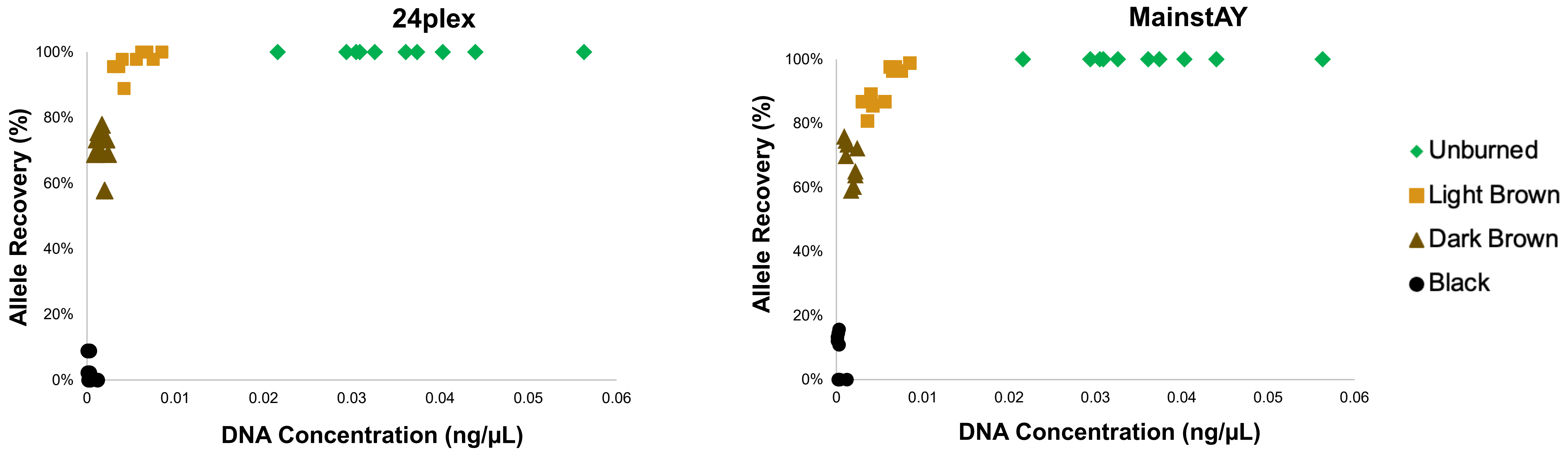
- Dark Brown and Black samples recovered similar concentrations of DNA
  - However, Black samples had a greater variability in degradation values



STR Genotyping

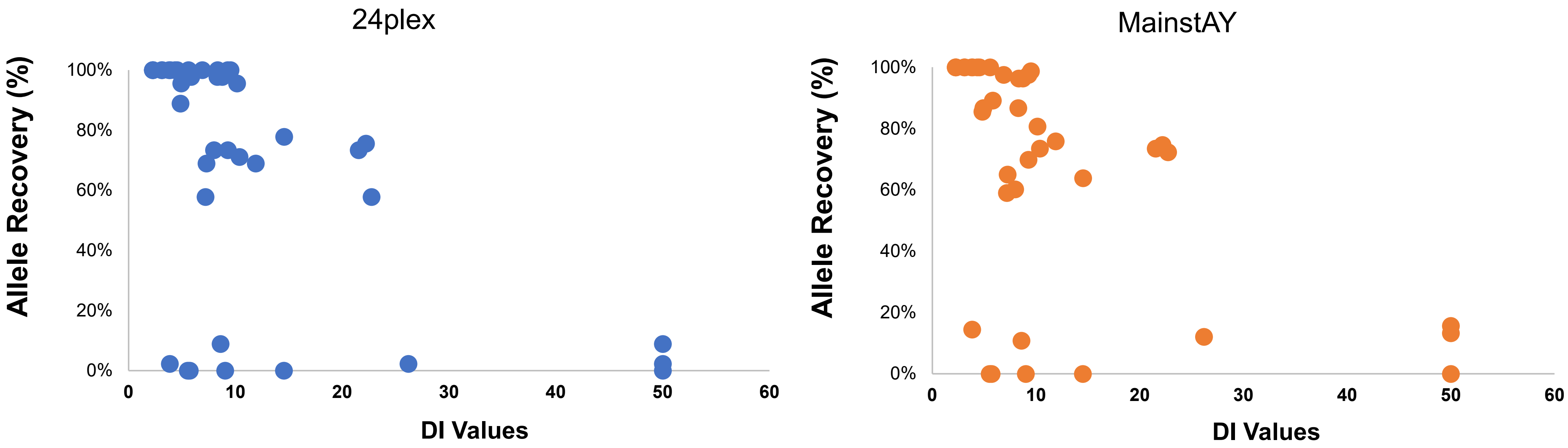
Investigator 24plex QS vs. ForenSeq MainstAY

Total Allele Recovery vs. DNA Concentration



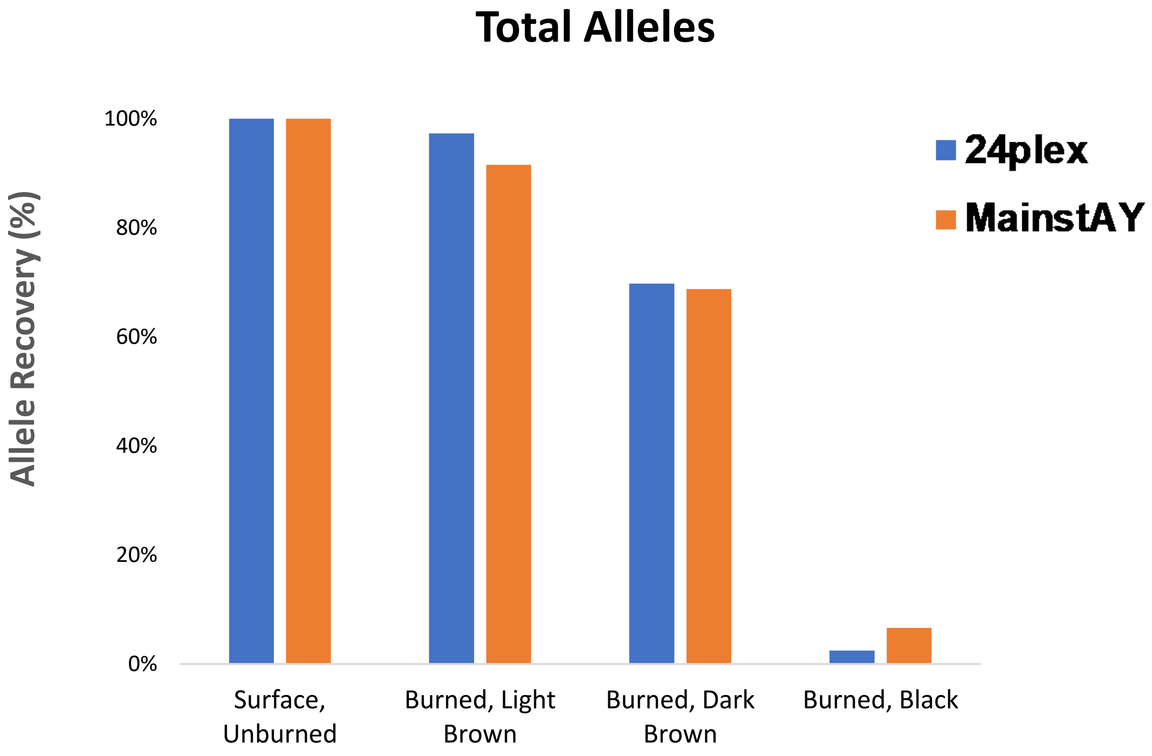
- As expected, more alleles were recovered with higher DNA concentrations, regardless of genotyping method (*24plex vs MainstAY*)
- Allele recovery was similar for both genotyping methods

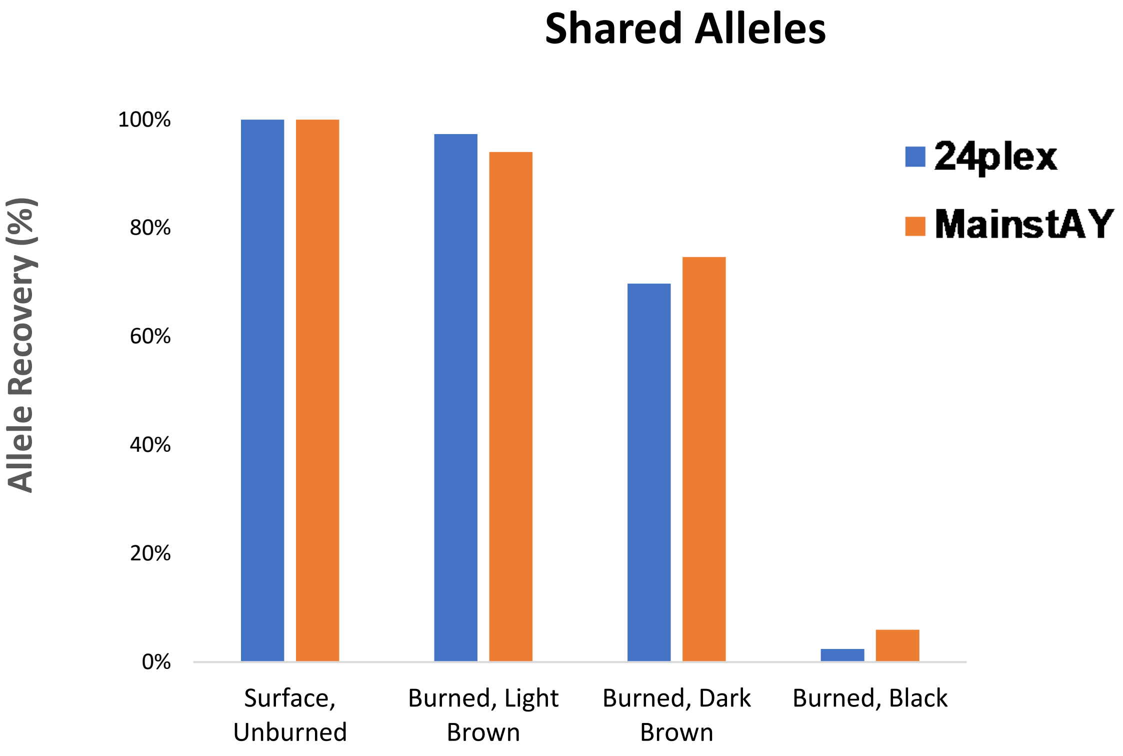
Total Allele % Recovery vs. Degradation Index



- Degradation values resulted in similar allele recovery for both genotyping methods
- No relationship was observed between DI value and number of concordant alleles recovered

Average % Allele Recovery - total and shared

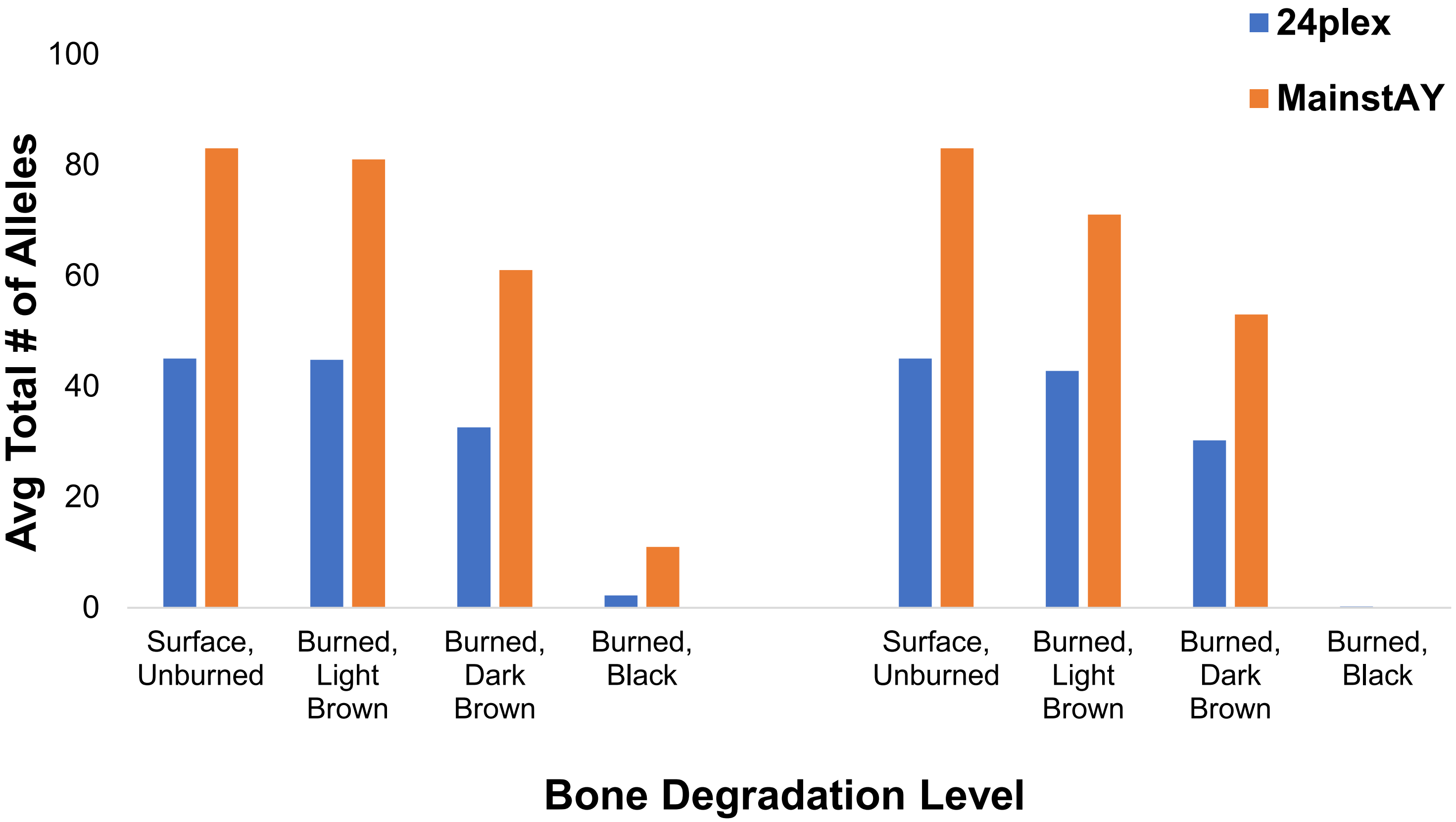




- Total 24plex Allele recovery was compared to both Total MainstAY alleles recovered (*left*) and those that are shared between the two kits (*right*)
- When comparing only the **shared** markers, average allele recovery increased slightly for both Burned, Dark Brown and Burned, Black samples with MainstAY

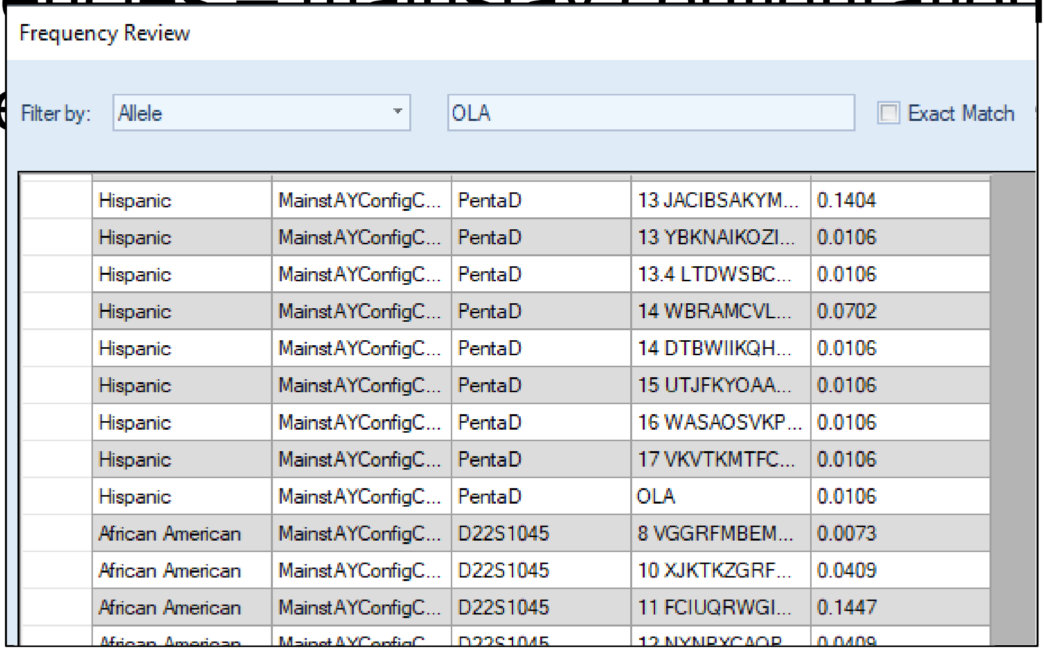
Average Number of Alleles Recovered

- When comparing total number of alleles recovered, MainstAY recovered a greater number of alleles for all sample types except Burned, Black
    - One cadaver yielded no alleles for any replicates using MainstAY and only 1 allele from one replicate using 24plex



What about Statistical Weighting?

- Software:
  - MixtureAce™ for MainstAY – NicheVision Forensics, LLC
  - ArmedXpert™ for 24plex – NicheVision
- Isoallele frequencies – mainstay configuration C
- Minimum allele le: 5/2N



Frequency Review

Filter by: Allele OLA ☐ Exact Match

Hispanic	MainstAYConfigC...	PentaD	13 JACIBSAKYM...	0.1404
Hispanic	MainstAYConfigC...	PentaD	13 YBKNAIKOZI...	0.0106
Hispanic	MainstAYConfigC...	PentaD	13.4 LTDWSBC...	0.0106
Hispanic	MainstAYConfigC...	PentaD	14 WBRAMCVL...	0.0702
Hispanic	MainstAYConfigC...	PentaD	14 DTBWIIKQH...	0.0106
Hispanic	MainstAYConfigC...	PentaD	15 UTJFKYOAA...	0.0106
Hispanic	MainstAYConfigC...	PentaD	16 WASAOSVKP...	0.0106
Hispanic	MainstAYConfigC...	PentaD	17 VKVTKMTFC...	0.0106
Hispanic	MainstAYConfigC...	PentaD	OLA	0.0106
African American	MainstAYConfigC...	D22S1045	8 VGGRFMBEM...	0.0073
African American	MainstAYConfigC...	D22S1045	10 XJKTKZGRF...	0.0409
African American	MainstAYConfigC...	D22S1045	11 FCIUQRWGI...	0.1447
African American	MainstAYConfigC	D22S1045	12 NYNBXCAOR	0.0409

Two things yet to solve

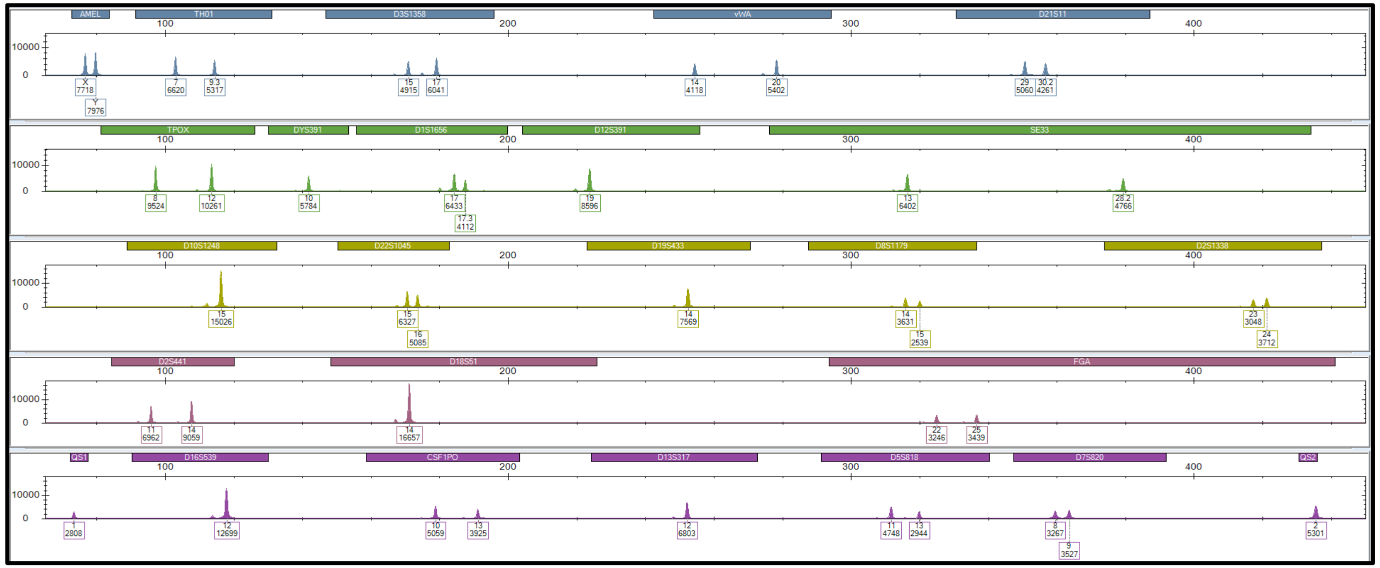
- Stochastic thresholds?
  - Unknown yet for NGS
  - (Need tweak to 1-click RMP stat to use Allele, Any genotype)

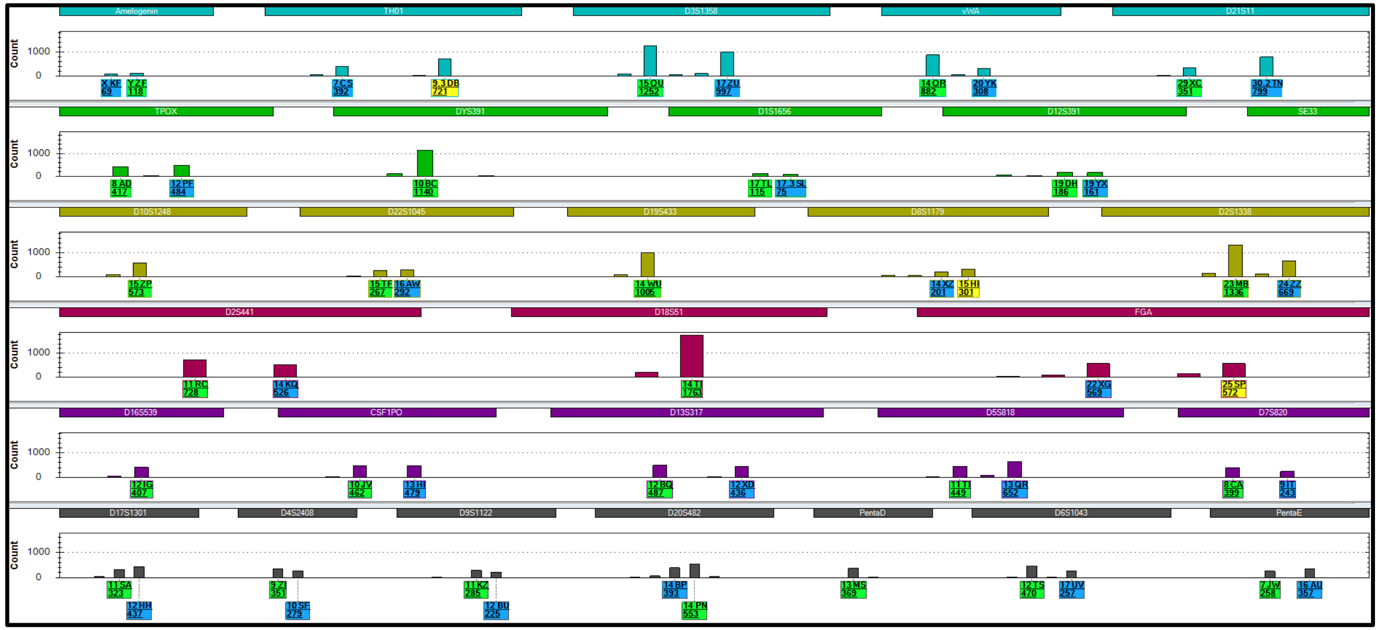
- Minimum Allele frequency
  - RMP traditionally uses 5/2n where n = number of people; 5/N
  - STRmix introduced us to 1/k+1 prior

$$\frac{x+\frac{1}{(k+1)}}{N+1}$$

x = allele count of allele x (x is allele of interest)
k = “buckets” of alleles seen at the locus (count of *different* alleles)
N = number of alleles in the database at the locus

Unburned - High level profile





Kit	AA	ASN	CAU	HIS
MainstAY	1 in 1.82E+56	1 in 1.08E+50	1 in 8.41E+51	1 in 4.20E+50
MainstAY: 24plex Only	1 in 4.93E+41	1 in 3.38E+37	1 in 2.47E+38	1 in 1.93E+37
24 Plex	1 in 2.20E+32	1 in 3.78E+31	1 in 1.25E+30	1 in 7.55E+29
24 Plex – SE33	1 in 2.33E+29	1 in 1.50E+29	1 in 3.17E+27	1 in 8.70E+26

56 zeroes = 180 Septendecillion
32 zeroes = 219 Nonillion

BR13_R1 Mid level profile

Kit	AA	ASN	CAU	HIS
MainstAY	9.93 E+39	3.52 E+35	8.77 E+37	5.81 E+36
MainstAY: 24plex Only	3.38 E+30	4.21 E+27	4.46 E+28	3.95 E+27
24 Plex	7.57 E+19	8.24 E+18	6.02 E+17	1.67 E+17
24 Plex – SE33	8.02 E+16	3.27 E+16	1.52 E+15	ADD

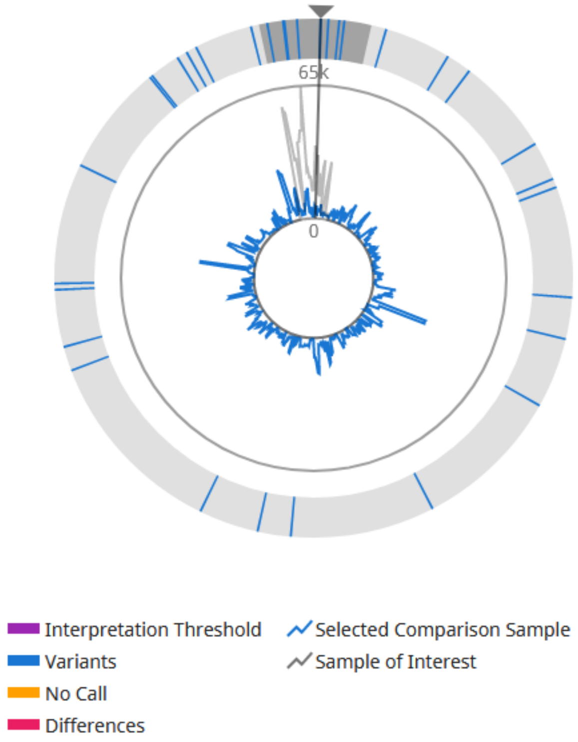
39 zeroes = 9.93 Duodecillion
19 zeroes = 75.6 Quintillion

BL22_R1 Low level profile

Kit	AA	ASN	CAU	HIS
MainstAY	1 in 7.55E+8	1 in 2.07E+7	1 in 2.65E+8	1 in 1.11E+8
MainstAY - 24 Only	1 in 3.60E+8	1 in 1.04E+7	1 in 9.45E+7	1 in 5.19E+7
24 Plex	1.34E+3	2.10E+3	721	ADD
24 Plex - SE33	1.34E+3	2.10E+3	721	ADD

8 zeroes = 755 Million
3 zeroes = 1,335

ForenSeq mtDNA Whole Genome Kit



Subset of light brown, brown, and black samples were processed with whole mtDNA kit

Run 1 Results – Cadaver 1

Sample	Insult	Human Quant (ng/µL)	DI	Reads
Light Brown – R2	Burned, Light Brown	0.0085	9.52	528,419
Light Brown – R3	Burned, Light Brown	0.0075	8.75	375,023
Light Brown – R4	Burned, Light Brown	0.0068	6.9	361,969
Light Brown – R5	Burned, Light Brown	0.0062	9.28	419,914
Brown – R1	Burned, Dark Brown	0.0009	11.89	471,284
Brown – R2	Burned, Dark Brown	0.0011	22.2	562,881
Brown – R3	Burned, Dark Brown	0.0013	10.38	443,203
Brown – R4	Burned, Dark Brown	0.0013	21.53	448,070
Black – R1	Burned, Black	0.0001	Und.	433,401
Black – R2	Burned, Black	0.0003	Und.	374,173
Black – R3	Burned, Black	0.0002	3.85	385,297
Black – R5	Burned, Black	0.0003	8.59	317,394

- All concordant calls – T1a1 Haplogroup

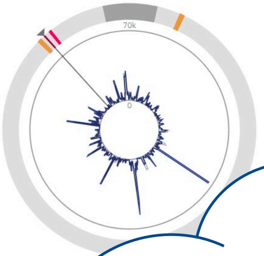
Run 2 Results – Cadaver 2

Sample	Insult	Human Quant (ng/µL)	DI	Reads
Light Brown – R2	Burned, Light Brown	0.0056	8.3	348,100
Light Brown – R3	Burned, Light Brown	0.0042	4.84	267,780
Light Brown – R4	Burned, Light Brown	0.004	5.84	359,041
Light Brown – R5	Burned, Dark Brown	0.0036	10.15	508,327
Brown – R1	Burned, Dark Brown	0.0022	7.27	451,900
Brown – R2	Burned, Dark Brown	0.0024	22.73	242,487
Brown – R3	Burned, Dark Brown	0.002	7.99	421,906
Brown – R4	Burned, Black	0.0022	14.55	446,700
Black – R2	Burned, Black	0.0003	14.53	1,908
Black – R3	Burned, Black	0.0002	Und.	1,285
Black – R4	Burned, Black	0.0012	5.53	2,343
Black – R5	Burned, Black	0.0004	5.71	1,735

- Concordant calls for light brown and brown – T2b6b Haplogroup

QIAxcel Connect Insights

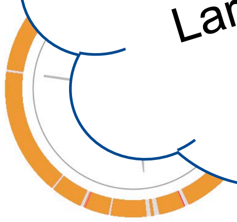
- DNA Quantity and Degradation Index were not predictive of mtDNA success
- Extracts were checked for quantity/quality on QIAxcel



Bla 1

Huma	s
	01

Quantiplex Pro DI:
Small Target: **91 bp**
Large Target: **353 bp**



: Run 2

Human Quant (ng/µL)	DI	Reads
0.0003	14.53	🟥 1,908

Black Bone – Run 1: Successful mtDNA Typing

			X-Small	Small	Medium	Large	X-Large	DI S-L	DI S-M	DI XS-S	DI M-L
Pos	Sample Information	Total Concentration [pg/µl]	15-75 bp	75-200 bp	200-600 bp	600-1000 bp	1000-3000 bp	Ratio (Conc.)	Ratio (Conc.)	Ratio (Conc.)	Ratio (Conc.)
E11	BL22-R1	211.47	84.42	43.16	58.83	19.06	121.76	2.26	0.73	1.96	3.09

Black Bone – Run 2: Unsuccessful mtDNA Typing

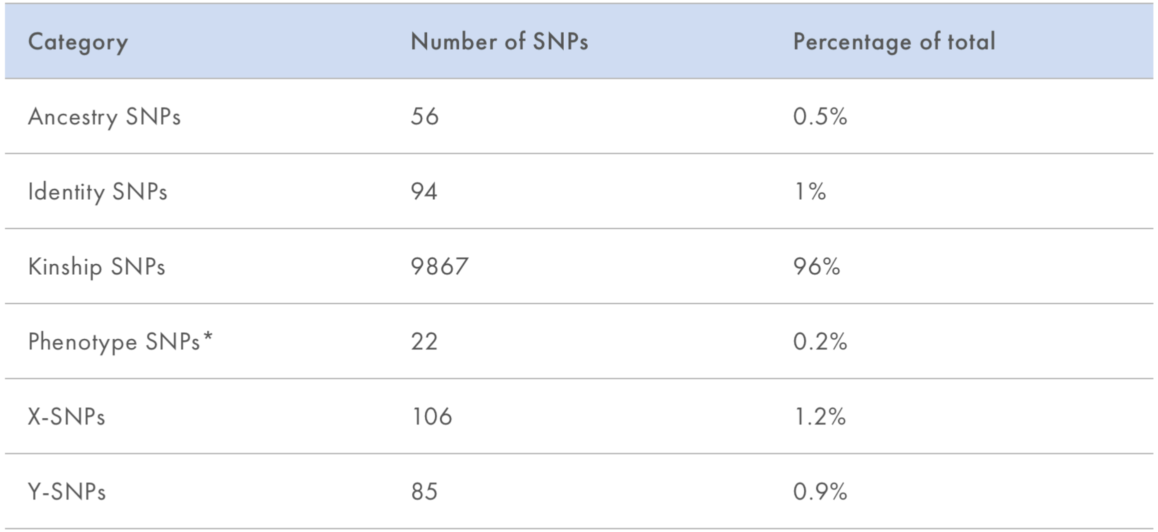
			X-Small	Small	Medium	Large	X-Large	DI S-L	DI S-M	DI XS-S	DI M-L
Pos	Sample Information	Total Concentration [pg/µl]	15-75 bp	75-200 bp	200-600 bp	600-1000 bp	1000-3000 bp	Ratio (Conc.)	Ratio (Conc.)	Ratio (Conc.)	Ratio (Conc.)
G9	BL17-R2	7.07	65.70	1.40	3.30	n/a	69.77	n/a	0.43	46.85	n/a

Summary

Next Steps

1. Perform Kintelligence on subset of burned remains
    - Dark brown and black samples

Average amplicon size of < 150 bp



Category	Number of SNPs	Percentage of total
Ancestry SNPs	56	0.5%
Identity SNPs	94	1%
Kinship SNPs	9867	96%
Phenotype SNPs*	22	0.2%
X-SNPs	106	1.2%
Y-SNPs	85	0.9%

2. Explore the predictiveness of the QIAxcel Connect

Acknowledgements

Thank You!

- American Academy of Forensic Sciences
- QIAGEN – Keith Elliott and Bryan Davis
- Niche Vision Forensics, LLC – Dr. Brian Young
- The Southeast Texas Applied Forensic Science Facility (STAFS), the donors, and their loved ones, without whom this research would not be possible
- Department of Forensic Science at SHSU
- Dr. Tim Kalafut

Questions?

Rachel Houston, PhD

Rachel.Houston@shsu.edu