Using the Ion S5™ and MiSeq FGx™ Systems to Identify Challenging Human Remains

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Disclaimer

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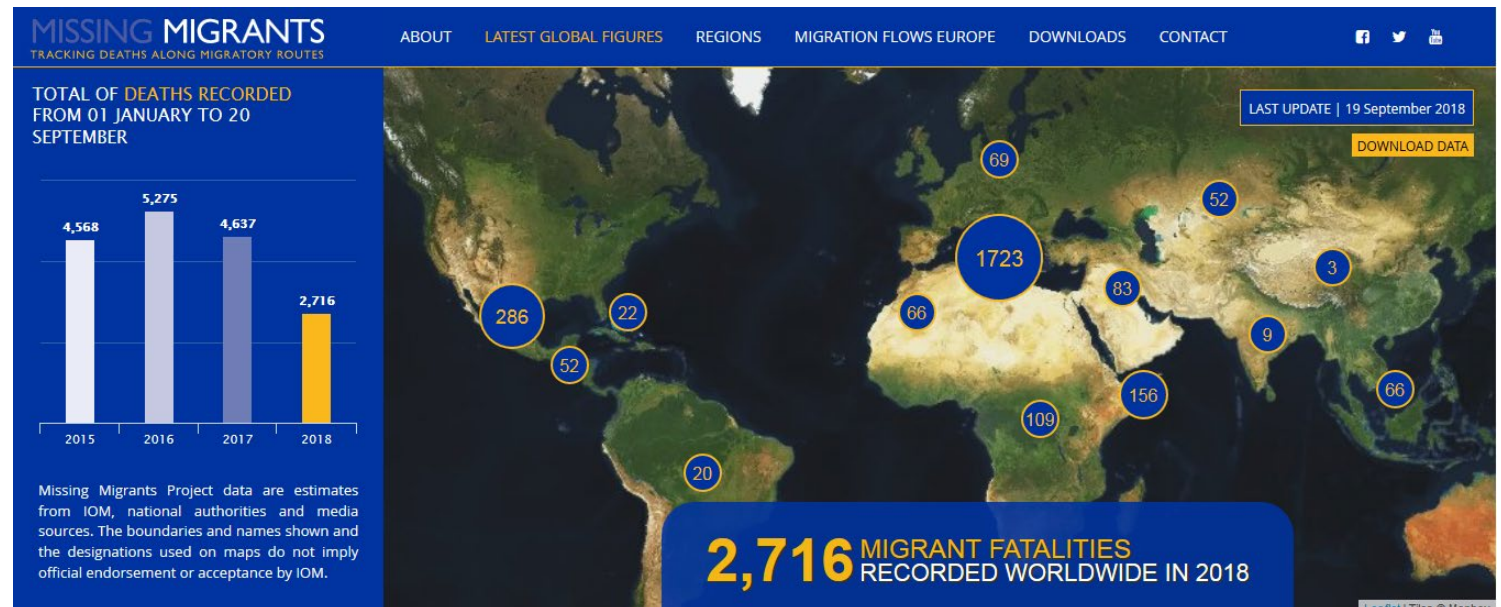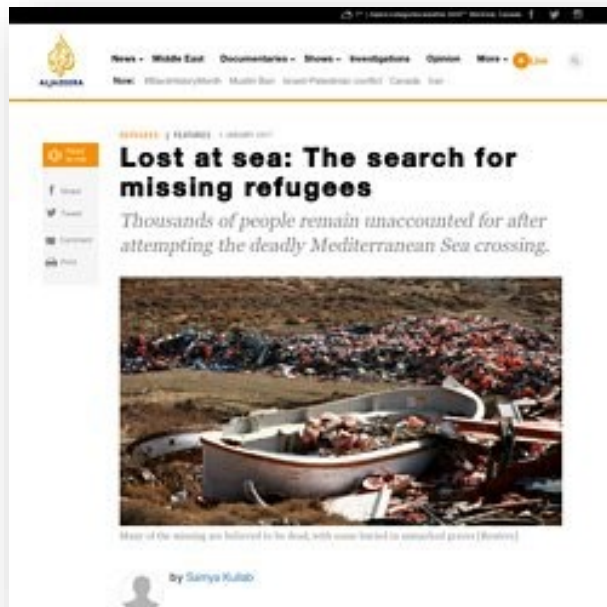
Introduction

Missing Person Cases

- Missing persons' cases, unidentified human remains, and mass disasters are problems faced worldwide
 - Migrants and refugees have died or gone missing in their efforts to cross seas and borders

Human Remains

- Skeletal remains (bone and teeth) are often the only samples available for DNA analysis

- Some samples are more challenging to process due to
    - Biological composition
    - Environmental exposure
    - DNA damage and/or degradation
    - Presence of inhibitors
    - Contamination/comingled remains

Current HID Methods

Globalfiler / Globalfiler Express

- **CE-based STRs**
  - Gold Standard
  - Multiplex capability
  - High PD
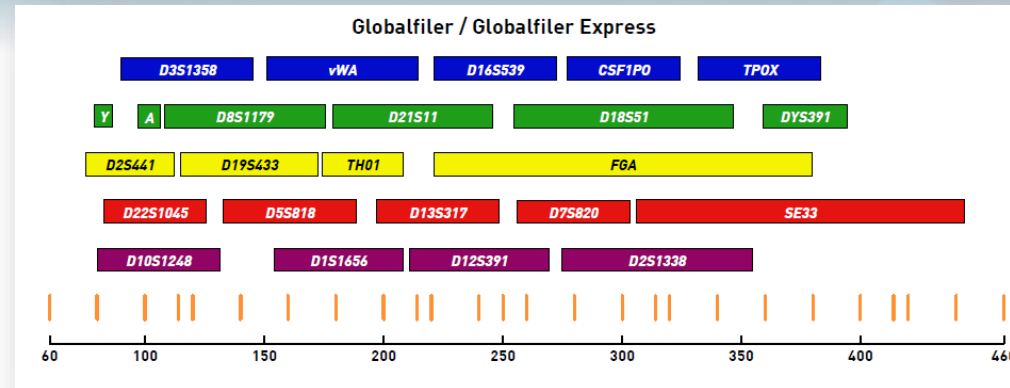
- **MPS**
  - Simultaneous analysis of different marker systems
    - STRs, iiSNPs, aiSNPs, piSNPs, microhaplotypes, mtDNA
  - Large multiplexing and increased throughput
  - Provides more genetic information
  - Detection of sequence variation
  - Mixture deconvolution

- **CE chemistries more mature; development refined for optimal sensitivity and tolerance**
 - Comparatively little for MPS platforms and chemistries

Materials & Methods

DNA Preparation

- Bone and Teeth
  - 14 donors
  - 24 samples
  - Various environmental insults

- Extracted with a total demineralization protocol
  - Loreille et al. 2010
  - Extracted in triplicate
  - 300 mg powder

- Quantifiler™ Trio DNA Quantification Kit
- GlobalFiler™ PCR Amplification Kit

| Substrate	Insult
Tooth	Thermally Degraded
Bone	Embalmed
	Cremated
	Burned
	Decomposed
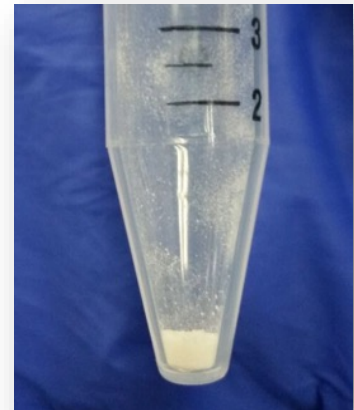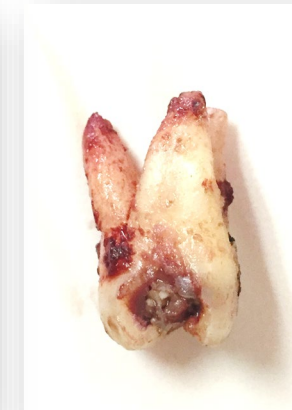
Ion S5™ Sequencing

- Precision ID DL8 and Library Kit
- Precision ID chemistry and a custom AmpliSeq™ STR and iiSNP primer panel
  - 32 STR markers
  - 1 Y-indel
  - 2 amelogenin sex markers
  - 41 iiSNPS
  - 34 Y-SNPs

- Ion Chef™ System

- Ion S5™ System
  - Torrent Suite Software v5.6
  - HID_SNP_Genotyper v5.2.2
  - Converge v2.0
  - In-house workbook

Ion S5™ Run Metrics

Chip	No. Samples	Pooling Concentration	% Chip Loading (40% - 70%)	% Usable Reads (>30%)	% Polyclonal (20% - 40%)	Total Reads	Mean Read Length
1	24	50 pM	42%	35%	34%	5,279,709X	78 bp
2	33	50 pM	56%	32%	34%	6,576,081X	112 bp
3	14	~26 pM	**36**%	**29**%	32%	3,748,684X	114 bp
4	28	~12 pM	**37%**	**28%**	30%	3,732,793X	102 bp
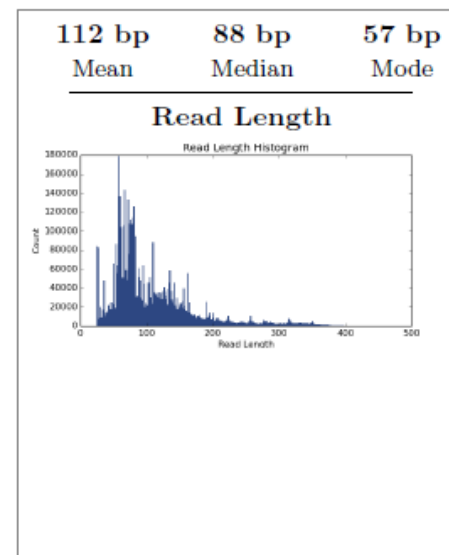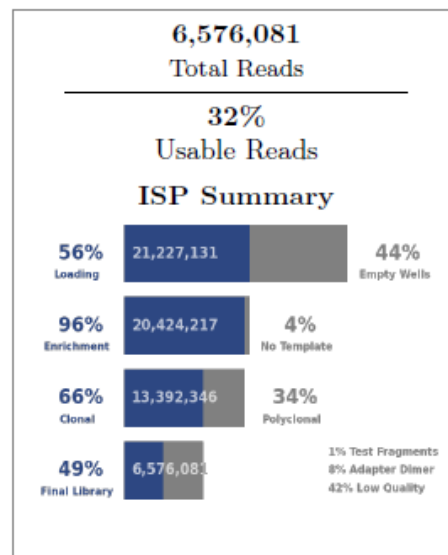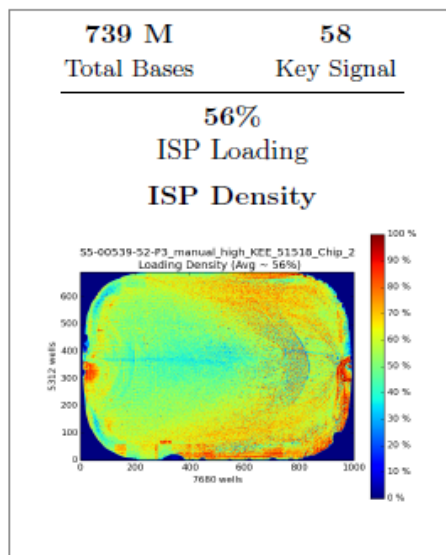
MiSeq FGx™ Sequencing

- ForenSeq™ DNA Signature Prep Kit
  - Primer Mix A
    - 27 autosomal STRs
    - 24 Y-STRs
    - 7 X-STRs
    - 94 iiSNPs

- MiSeq FGx ™ work performed at UNTHSC
  - Universal Analysis Software
  - STRait Razor v2s

MiSeq FGx™ Run Metrics

Run	No. Samples	Cluster Density (400-1650 K/mm^2)	Cluster Passing Filter (≥ 80%)	Phasing (≤ 0.25%)	Pre-phasing (≤ 0.15%)
1	32	642	93.81%	**0.285%**	-
1 re-run	24	**294**	97.62%	0.211%	-
2	31	1060	90.08%	0.160%	0.032%
3	32	1143	88.08%	0.153%	0.098%

RUN METRICS   SAMPLE REPRESENTATION

RUN QUALITY METRICS

Cluster Density

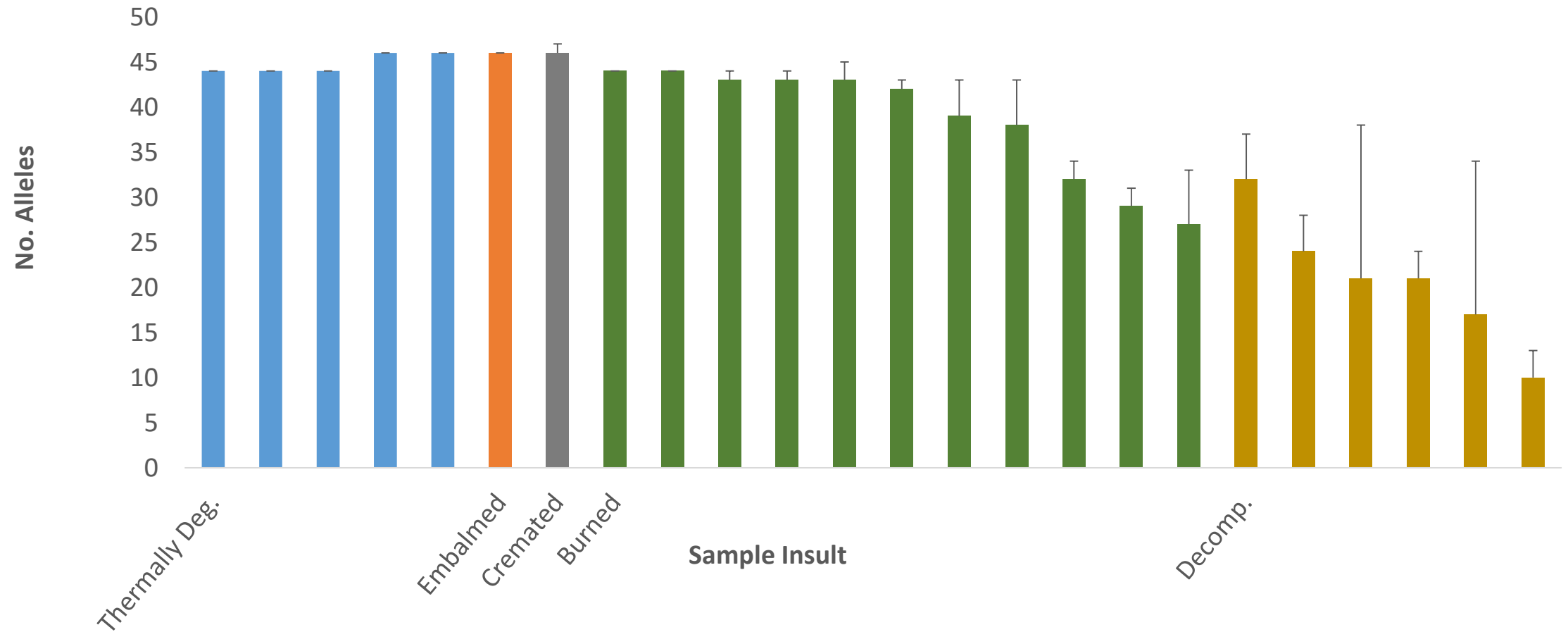1143 k/mm^2

Clusters Passing Filter

88.08%

Phasing

0.153%
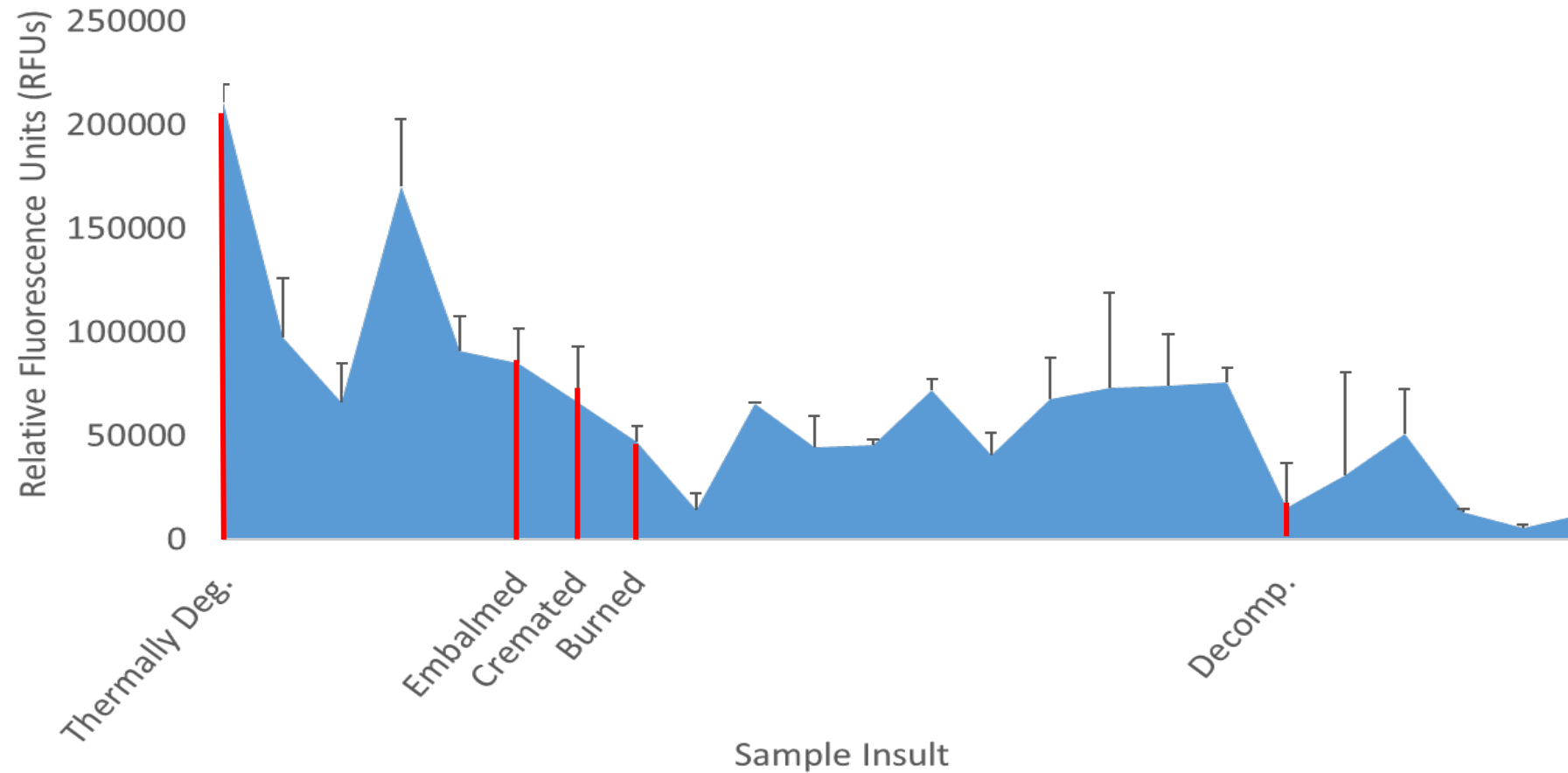
Pre-Phasing

0.098%

CE Results

Reportable Alleles

- Reportable STR alleles ranged from 10 ± 3 to full profiles (31%-100%)
- Thermally degraded, embalmed, and cremated samples produced the most complete profiles, decomposed samples resulted in the least amount of alleles
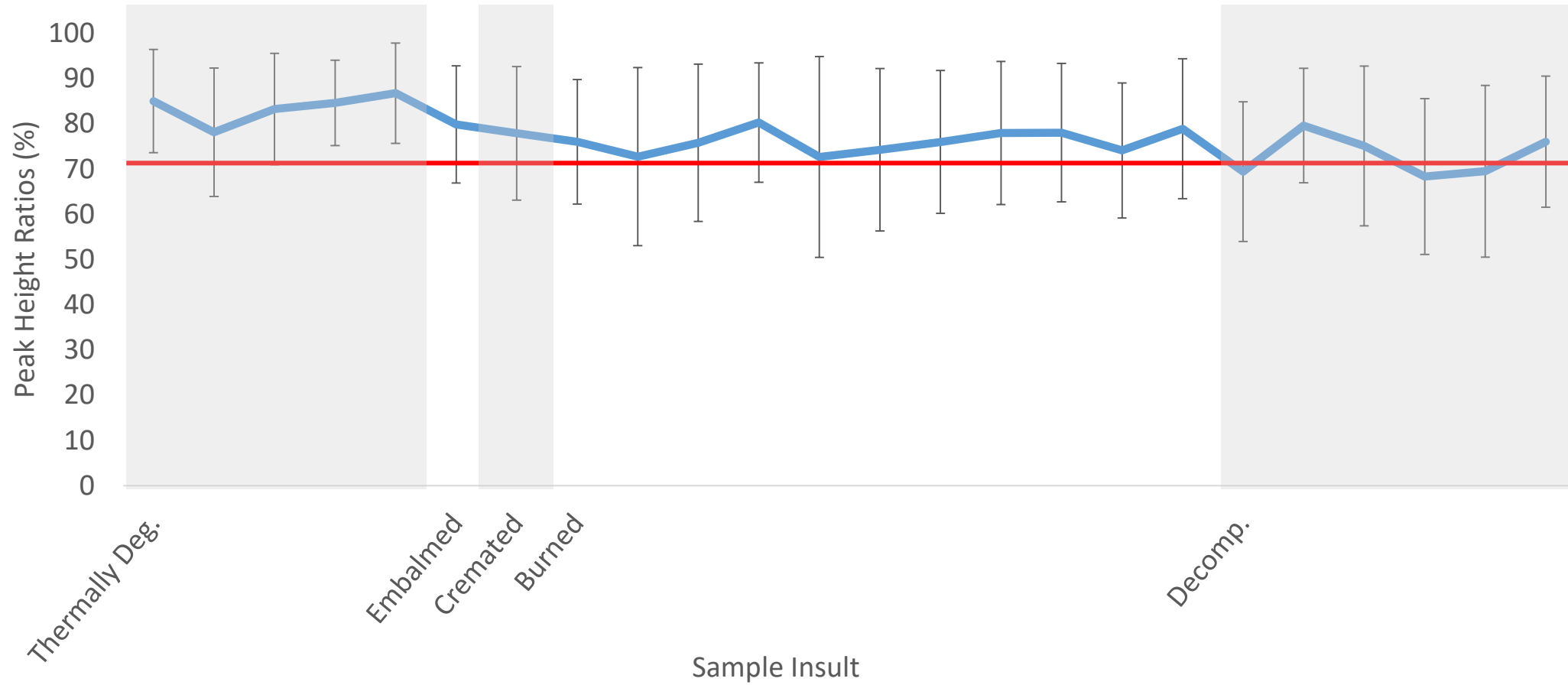
Peak Height

- Thermally degraded samples averaged the highest peak heights, followed by embalmed, cremated, and burned
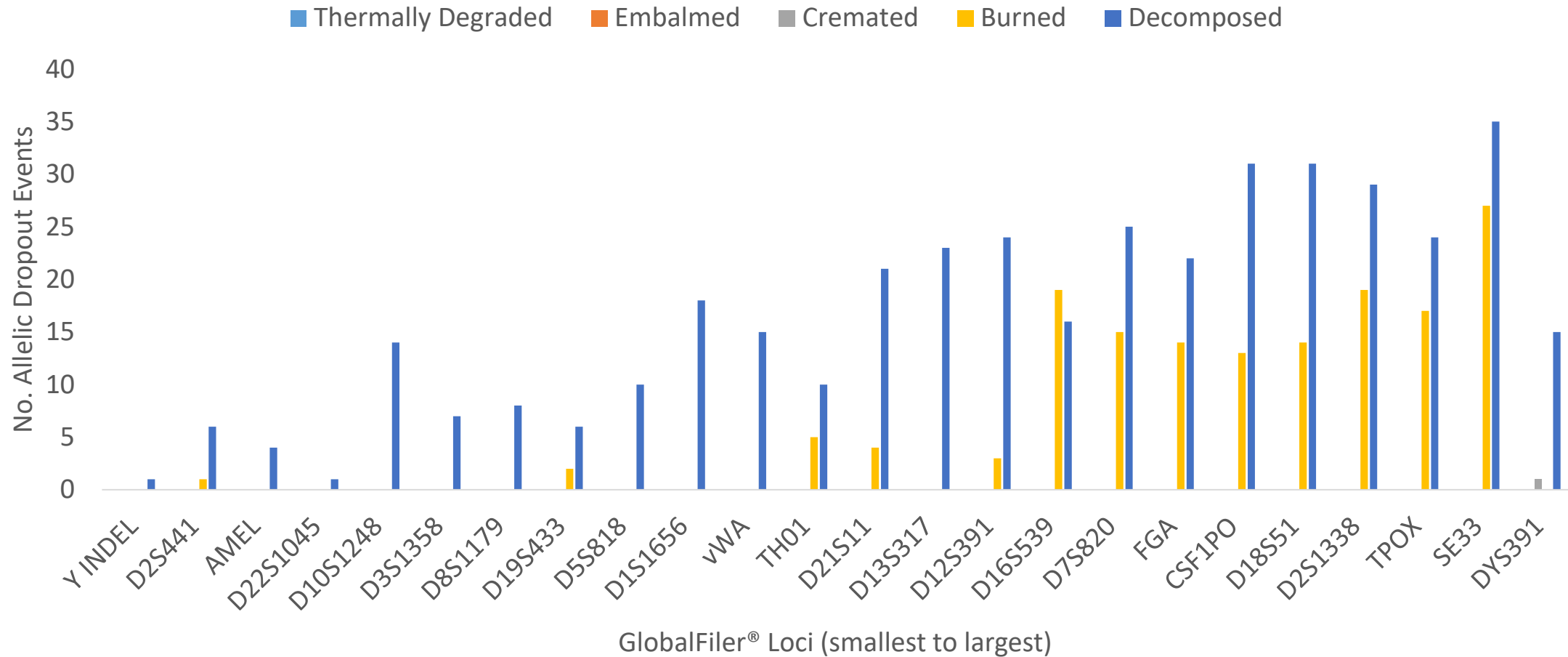- Decomposed bones produced the lowest average peak heights

Peak Height Ratios

- Peak height ratios ranged from 68%-87%
- Three samples (all decomposed) produced average PHRs <70%

Allelic Dropout

- Majority of dropout occurred in burned and decomposed samples
- 153 alleles dropped out with burned samples and 396 alleles dropped out in decomposed remains across all samples
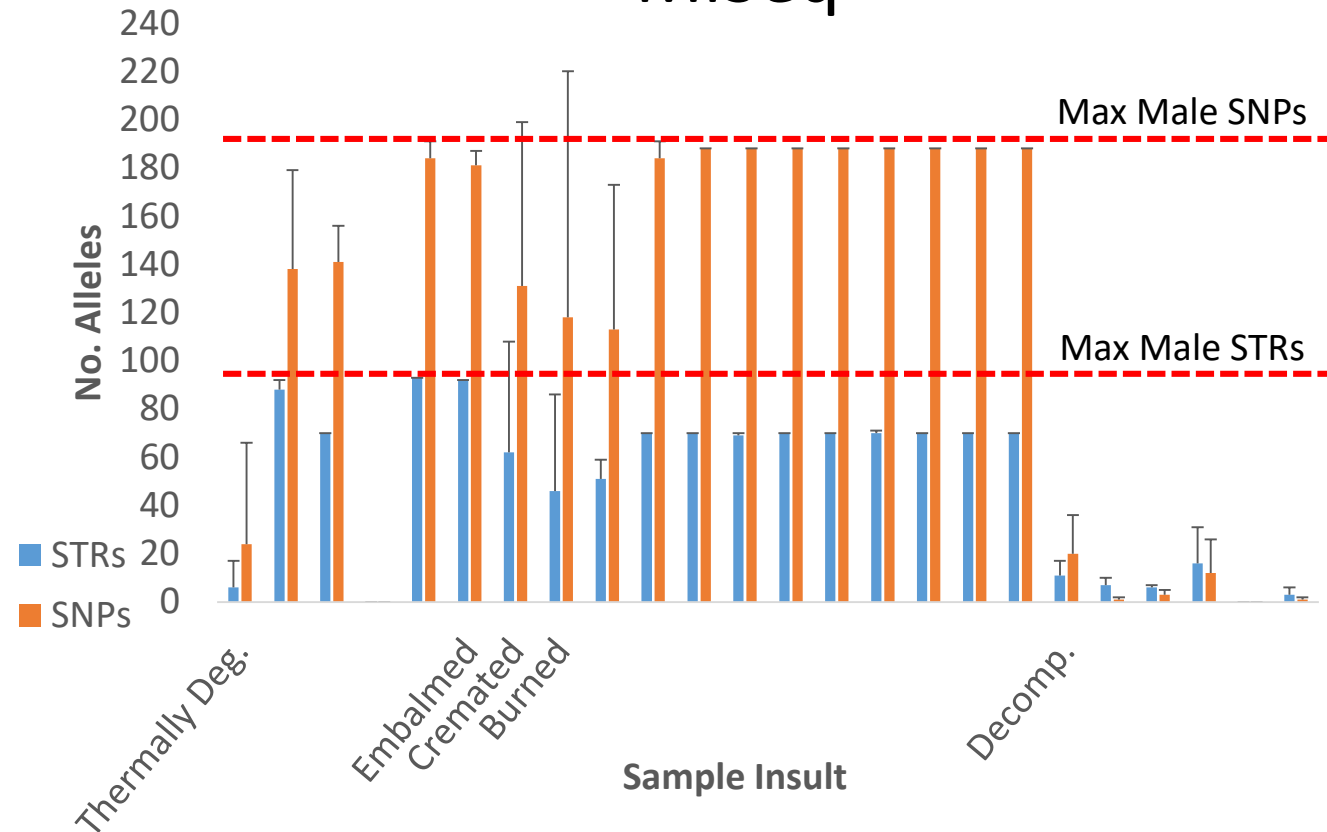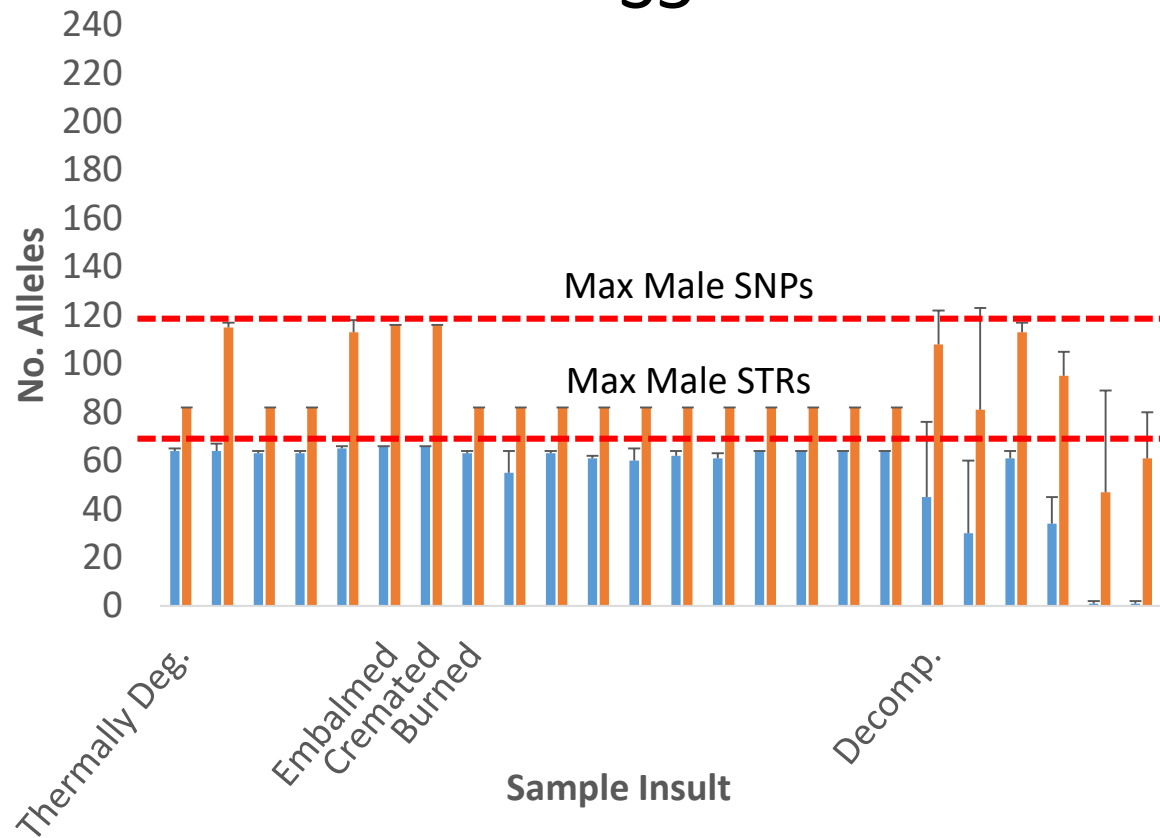- Larger loci dropped out more often

MPS Results

Reportable Alleles

S5 chart (left): axis "No. Alleles" 0–240, x-axis "Sample Insult" with labels Thermally Deg., Embalmed, Cremated, Burned, Decomp. Series: STRs (blue), SNPs (orange). Reference lines: Max Male SNPs (~120), Max Male STRs (~68).

MiSeq chart (right): axis "No. Alleles" 0–240, x-axis "Sample Insult" with labels Thermally Deg., Embalmed, Cremated, Burned, Decomp. Series: STRs, SNPs. Reference lines: Max Male SNPs (~192), Max Male STRs (~95).

- The Ion S5 profile completeness ranged from one allele to full profiles for STRs, and 61 alleles to full profiles for SNPs
  - All samples except decomposed remains produced >90% of alleles for STRs and SNPs
  - SNPs demonstrated higher profile completeness, ~93% vs ~84%
- For the MiSeq, STRs and SNPs produced profiles ranging from 0 alleles to full profiles
 - Profile completeness between STRs and SNPs was highly comparable, ~66% vs ~63%

Reportable Alleles - STRs
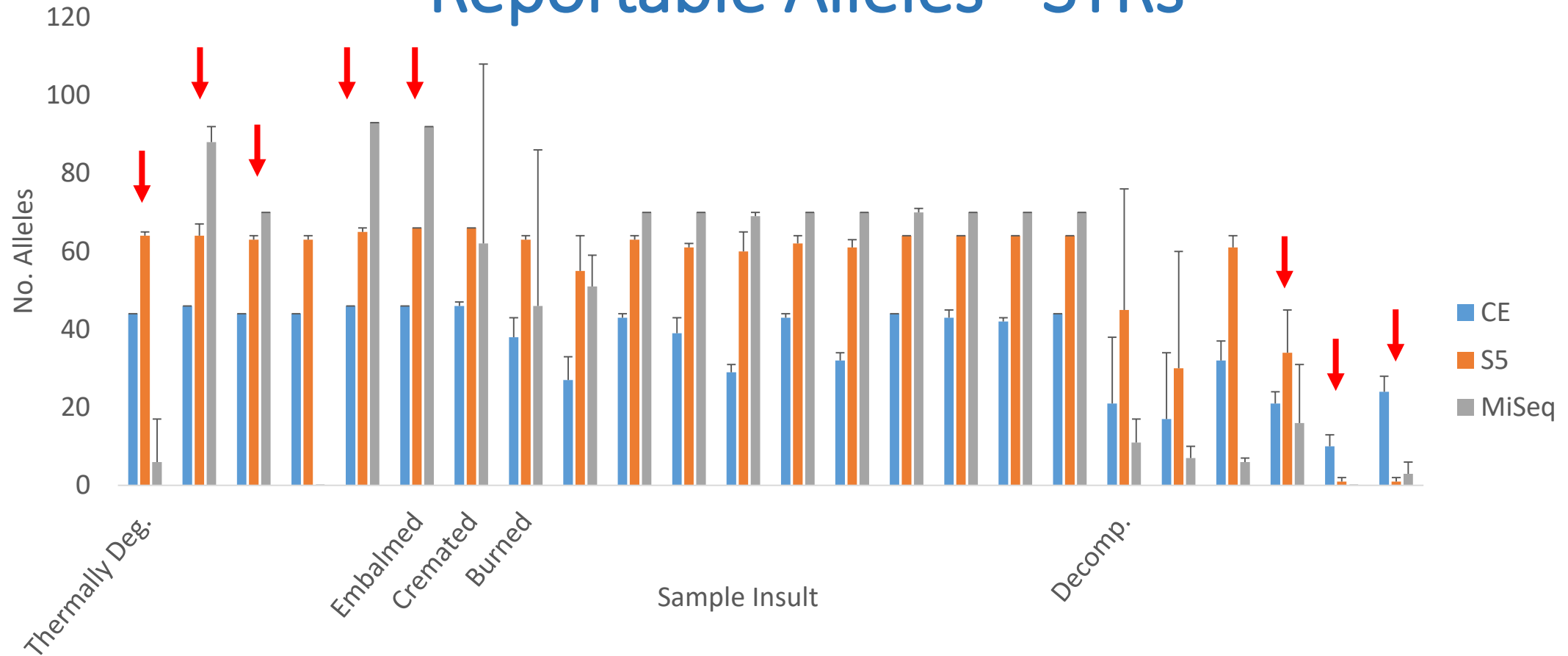
- For all but 2 samples, at least one of the MPS platforms produced more alleles than CE
- The S5 produced more alleles than the CE for 22/24 samples
- The MiSeq produced more alleles than the CE for 16/24 samples, but CE produced more alleles than the MiSeq for all decomposed remains
  - Less DNA template available for amplification - maximum sample input for the MiSeq is only 5 µL - ~50 pg

Reportable Alleles – CODIS Loci
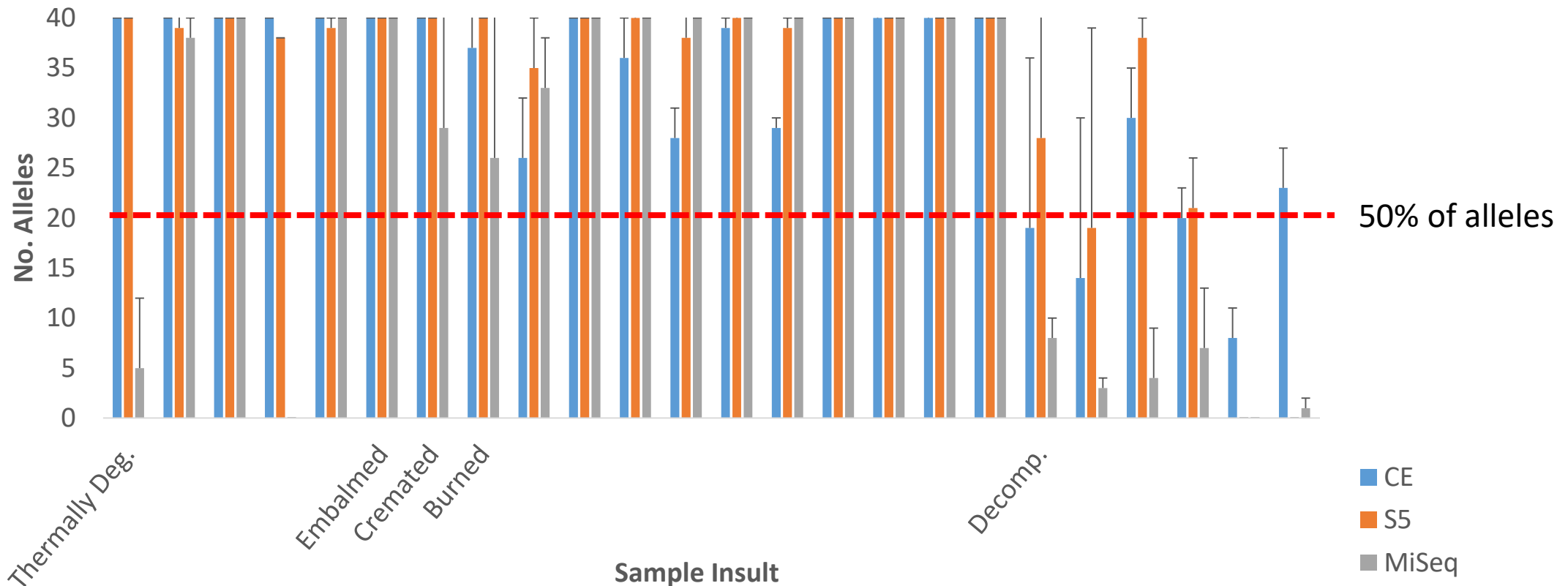
- CE produced 8 to 40 alleles, with all but 3 samples producing above 50% of alleles
- S5 and MiSeq profiles ranged from 0 to 40 alleles
  - 2 samples produced no profile when sequencing with the S5 and the MiSeq
  - CE produced 5 more complete profiles than the S5, and 11 CE samples with more alleles than the MiSeq
  - Overall, when combining sequencing platforms, only 4/24 CE samples produced more alleles
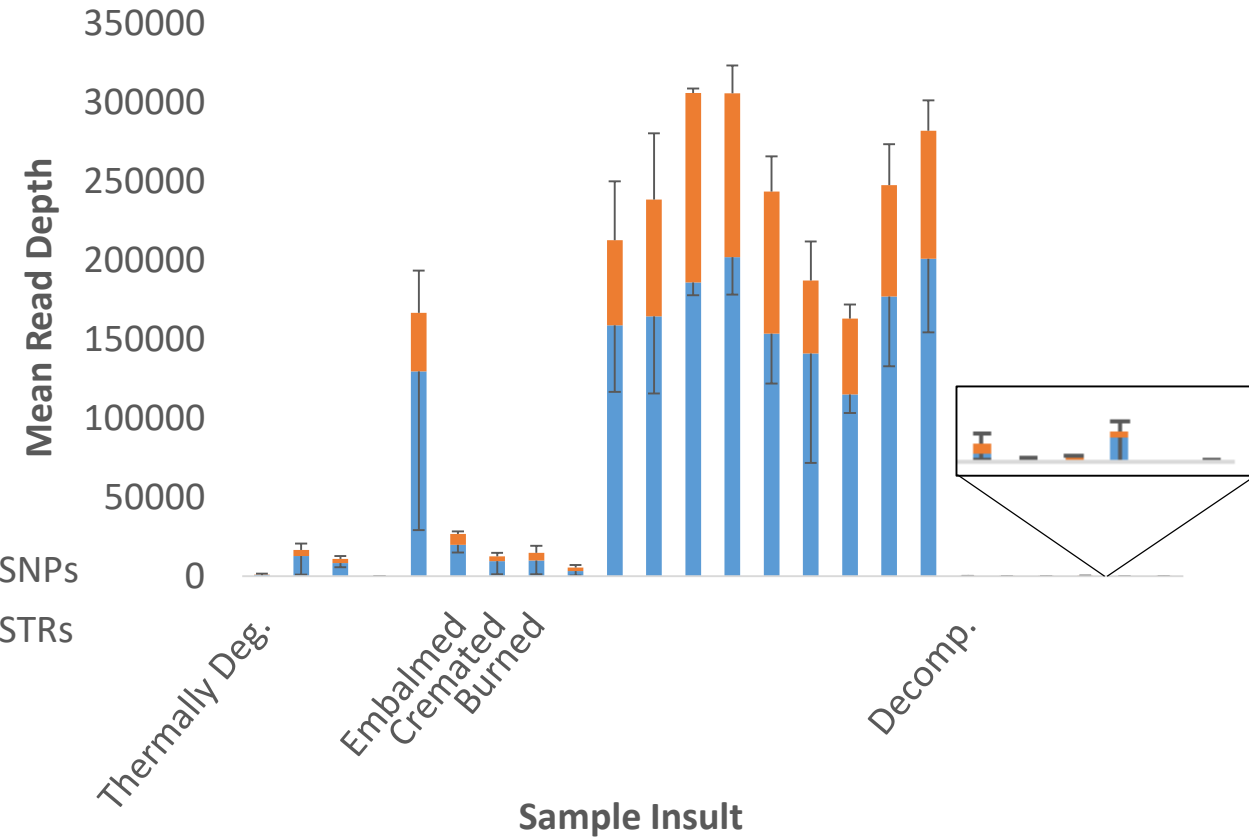- All three platforms produced 12/24 full profiles
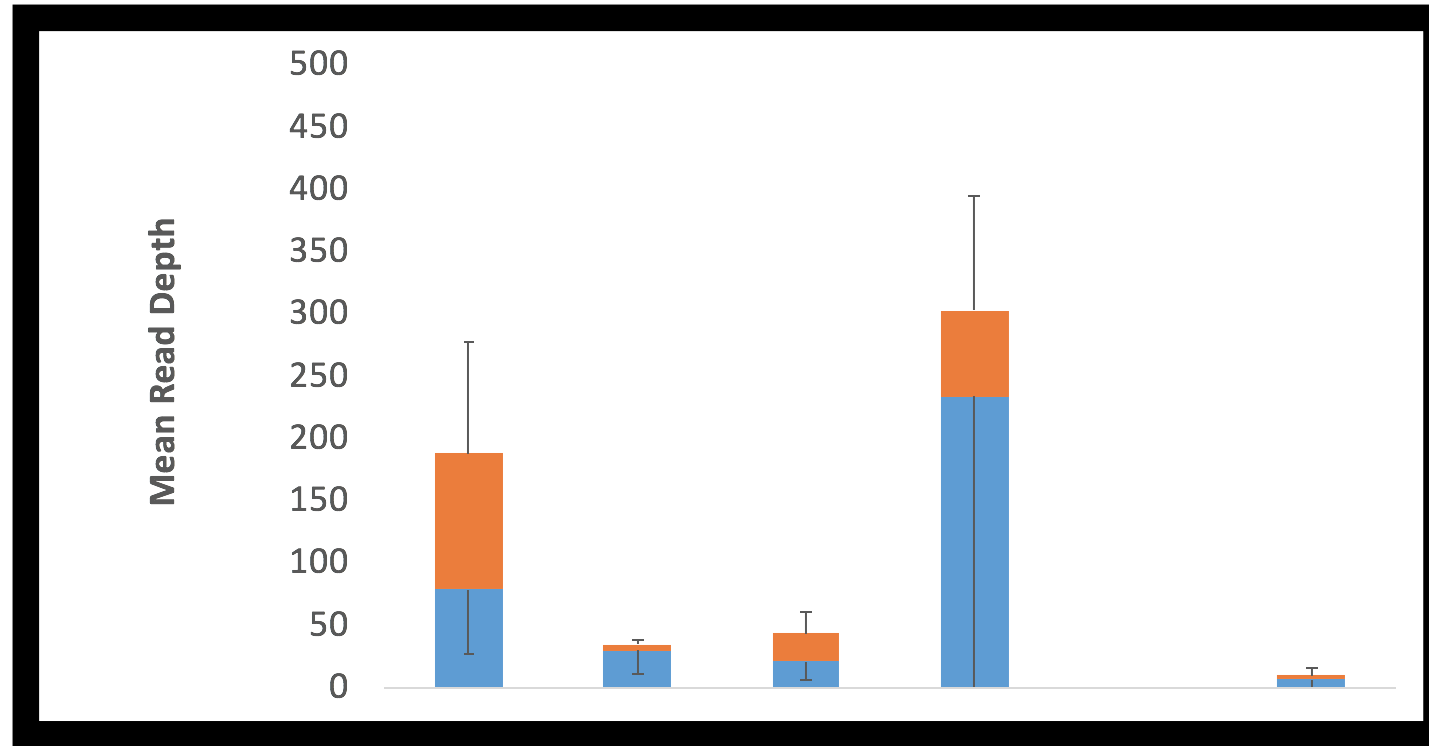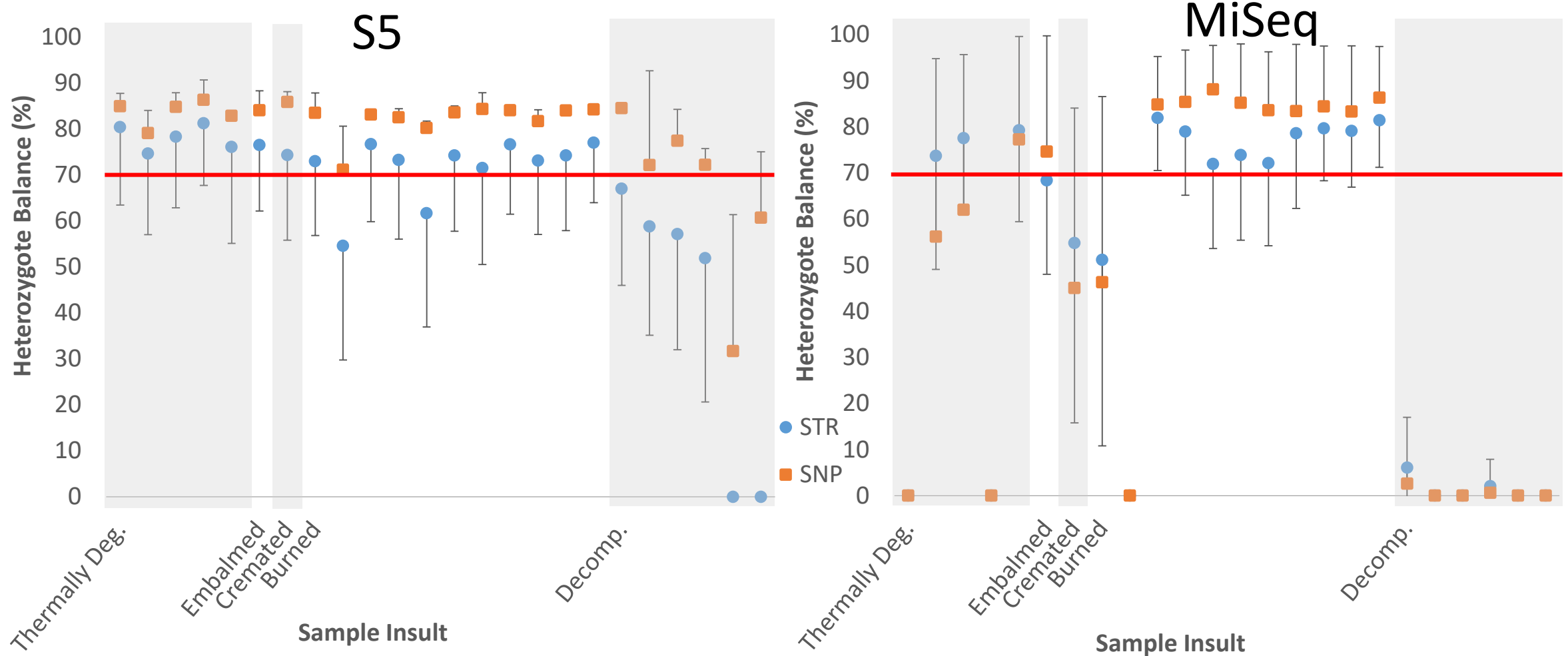
Mean Read Depth
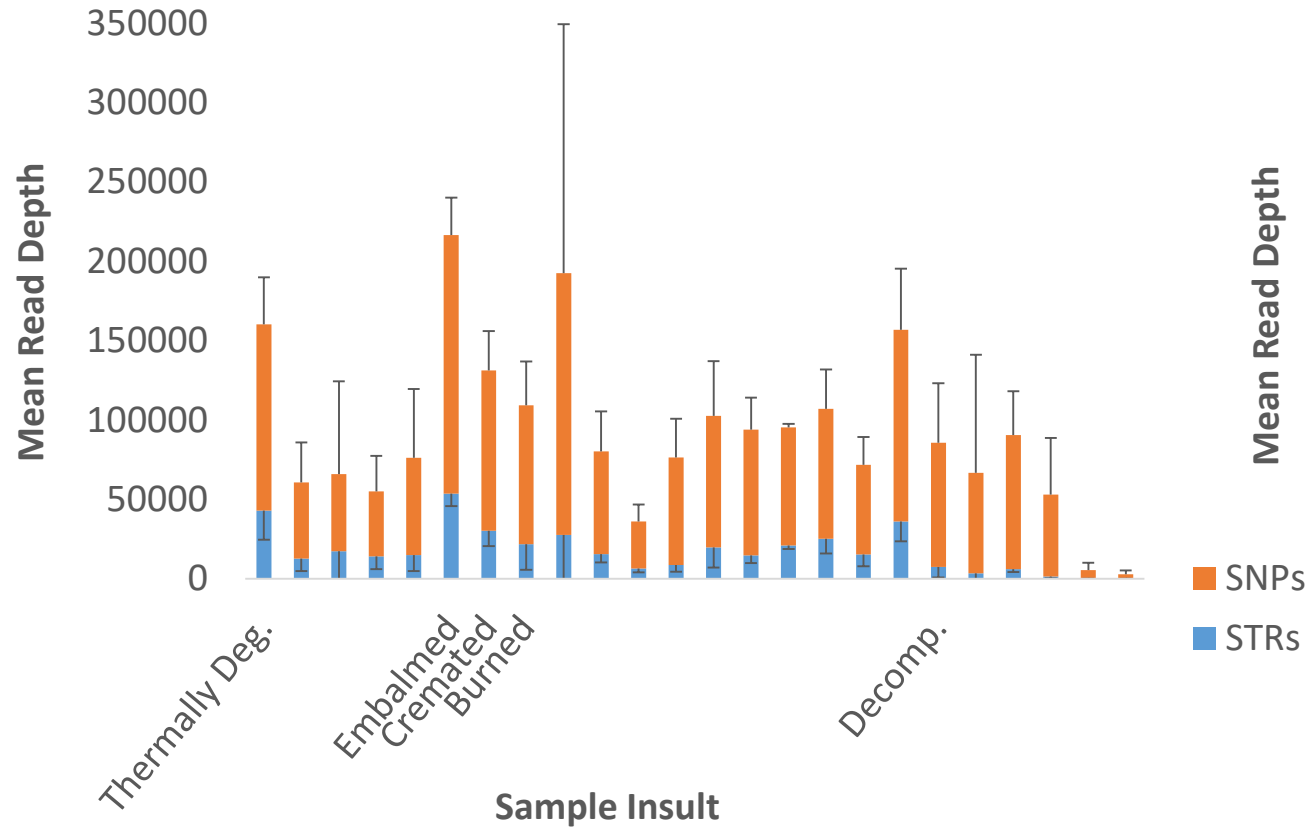
- S5 SNPs produced higher coverage than STRs for every sample, and both STRs and SNPs were well balanced
- MiSeq STRs produced higher mean read depth than SNPs for most samples
- The MiSeq demonstrated a large increase in coverage for burned samples

Mean Read Depth

MiSeq

Heterozygote Balance

- For the S5, most samples showed an average heterozygote balance >70% and balance was ~15% higher for SNPs than STRs
- For the MiSeq, half of the STR samples and over half of the SNPs demonstrated heterozygote balance <70%; however, burned samples showed good balance

Conclusions

Conclusions

- Challenging remains pose a problem for analysis, but using MPS can increase the amount of genetic information recoverable from these types of samples

- Some samples did fail to produce a profile using MPS, while all CE samples produced a profile
  - CE is still very valuable
  - Abundance of STR and SNP markers may make MPS more probative even if the percentage of CE markers is higher

- Decomposed human remains proved most challenging for each platform/chemistry (particularly the MiSeq)
  - However, most samples were compatible with both chemistries

Overall Outcome

Challenging remains can be difficult to process and analyze, but MPS may provide more information with higher powers of discrimination than CE-based analysis and therefore may identify more remains and solve more cases in the future

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Questions?

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