Comparison of DNA Yield & STR Success Rates from Different Tissues in Embalmed Bodies

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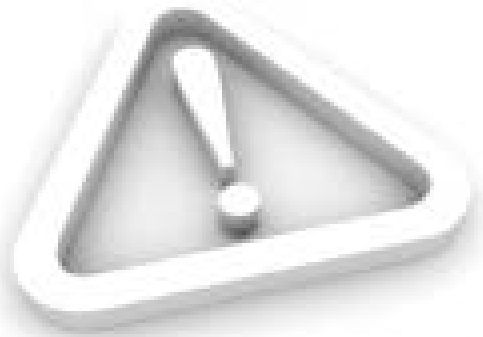
Disclosure

- There have not been any endorsement or financial relationship/interest from the following companies
  - Dodge Company: Introfiant & Chromatech Tan
  - Pierce Companies: Care 18
  - QIAGEN: Buffer ATL, Proteinase K, DTT, QIAamp® FFPE Tissue Kit
  - ThermoFisher: Quantifiler Trio® DNA Quantifiler Kit, Globalfiler® PCR Amplification Kit
- There have been no real or apparent conflicts of interest



WARNING

There is some content in this presentation that some people may find disturbing

Introduction

- When Formalin-Fixed or Formalin-Fixed Paraffin Embedded (FFPE) tissues are the only source of genetic material
  - Alternative sources of identification or diagnosis in unexplained deaths
  - Pathology and anatomical samples
  - Identification of exhumed embalmed bodies or human remains

Introduction

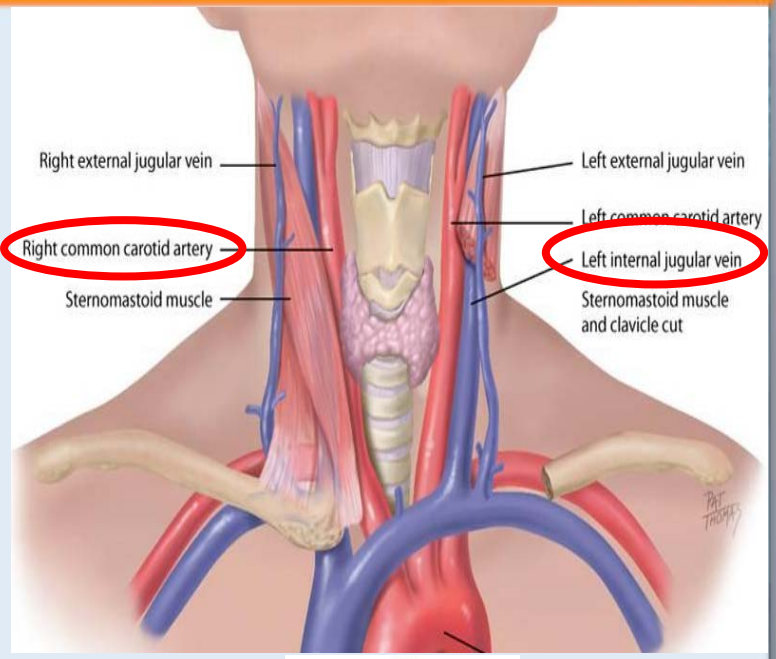
- Bones and teeth
  - Traditional sample of choice for DNA analysis with embalmed and skeletal remains
  - Soft tissue presumed too highly damaged and degraded
  - More difficult to collect, and labor intensive to process
  - Specialized, time consuming & costly extraction procedures
  - PCR inhibitors (calcium, collagen)

Soft tissue is easier to collect and process, but will STR typing for identification be successful?

Embalming Background

Formalin fixation

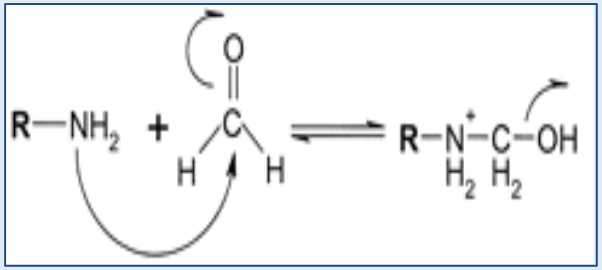
- Preservation of biological tissue sections and/or whole bodies for medical use or burial
  - Formalin: solution of formaldehyde (ranges from 5 – 35%) in water



- Solution injected via an embalming machine into the carotid artery → jugular vein
  - Also in the femoral artery

Embalming and DNA

- Fixation leads to protein – formaldehyde interactions
  - Carbonyl groups link with amine groups forming methylene bridges fixing the tissue



Higher concentration formalin → More methylene bridges formed → Nucleic acid fragmentation (200 – 300 bp)

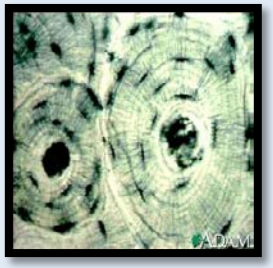
Problems with Embalming & DNA

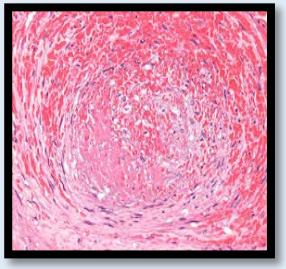
- DNA fragmentation makes it difficult to amplify high molecular weight DNA
  - Locus and allele drop out (partial profiles)
- PCR inhibitors (formaldehyde) may hinder DNA amplification
  - Direct interaction with DNA or interfering with DNA polymerase

Overall, formalin fixation decreases DNA quality and quantity

Embalming Fluid Distribution

- Exposure depends on the distribution of the chemicals
  - Density of capillaries in a tissue determines its exposure to the solution
    - Areas with high vascularity: muscle, internal organ & epidermis
    - Areas with low vascularity: bone, cartilage, hair & nails





More vascularized the tissue → Higher exposure to formalin → More DNA degradation → Decreased STR success

Embalming Fluid Distribution

- Livor Mortis: pooling of blood by gravity once the heart stops pumping
  - Tissues that are compressed will not show blood pooling (white)
  - Non-compressed tissues will (reddish)

Material & Methods - Cadavers

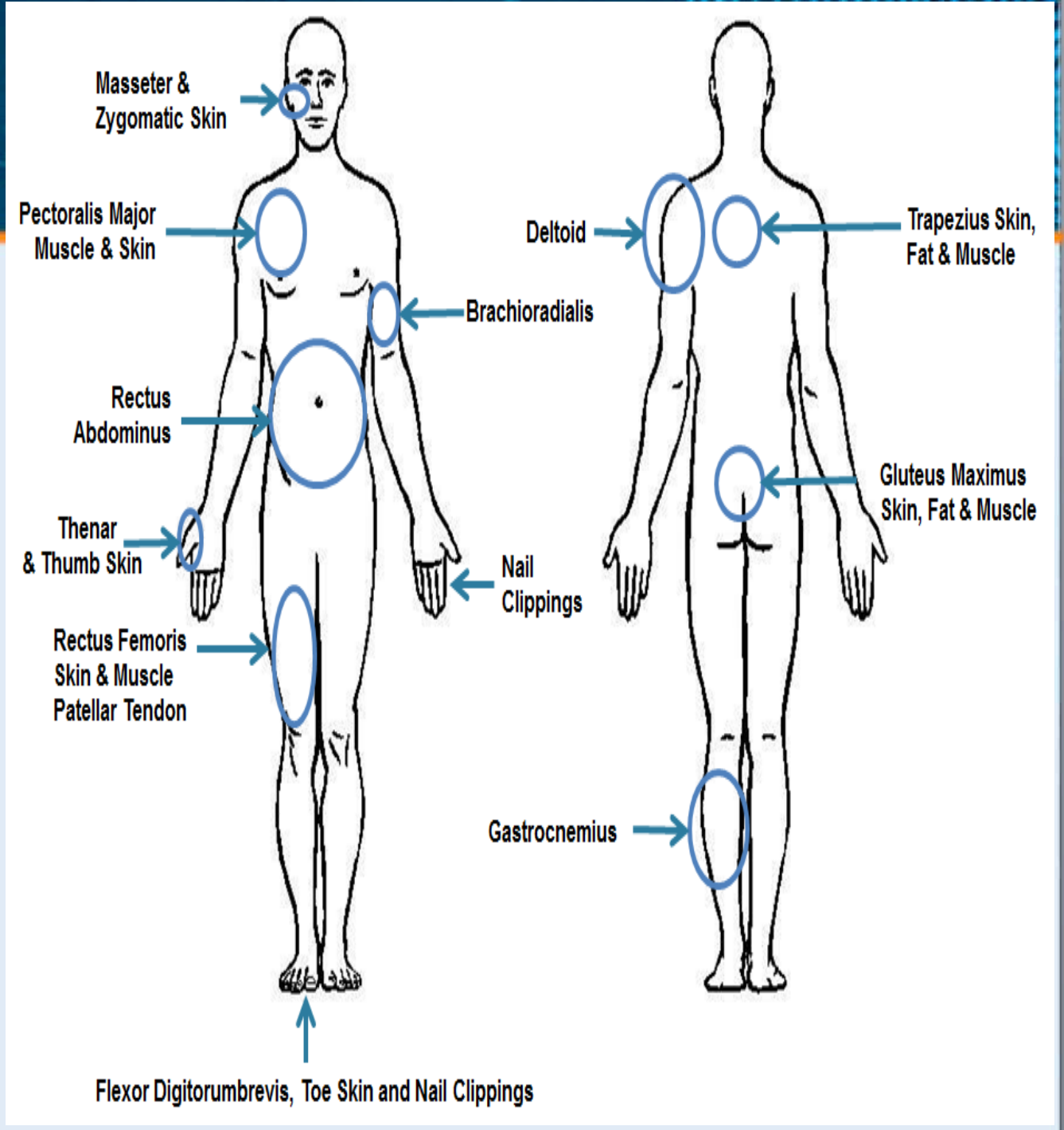
- Three male embalmed cadavers
  - Arterial injection of embalming fluid

Cadaver	Company	Embalming Fluid	Fixative	Amount
1	Dodge	Introfiant	20-50 % Formaldehyde	946mL
2	Dodge	Chromatech Tan	10-25 % Formaldehyde	473mL
3	Pierce	Care 18	15-25% % Formaldehyde	1400mL

- Tissue samples (N = 122)

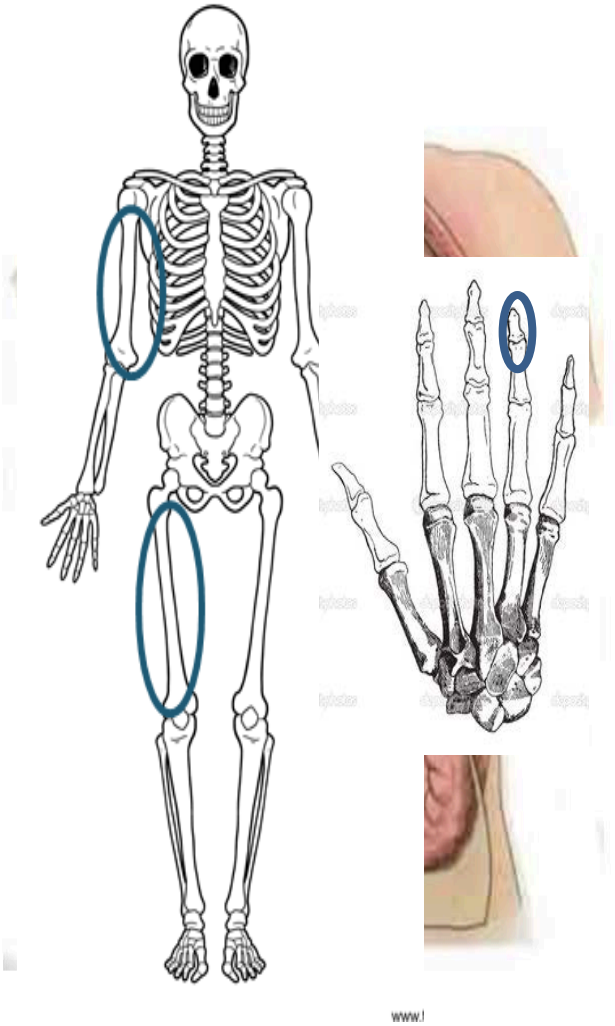
Skin, Fat & Muscle

- Also collected:
  - Psoas Major
  - Head Hair
  - Facial Hair
  - Pubic Hair



Internal Organs

- Brain (Gray Matter)
- Eye (Sclera)
- Left Lung
- Heart
- Jejunum
- Liver
- Kidney
- Stomach
- Spleen
- Patellar Tendon
- Calcaneal Tendon



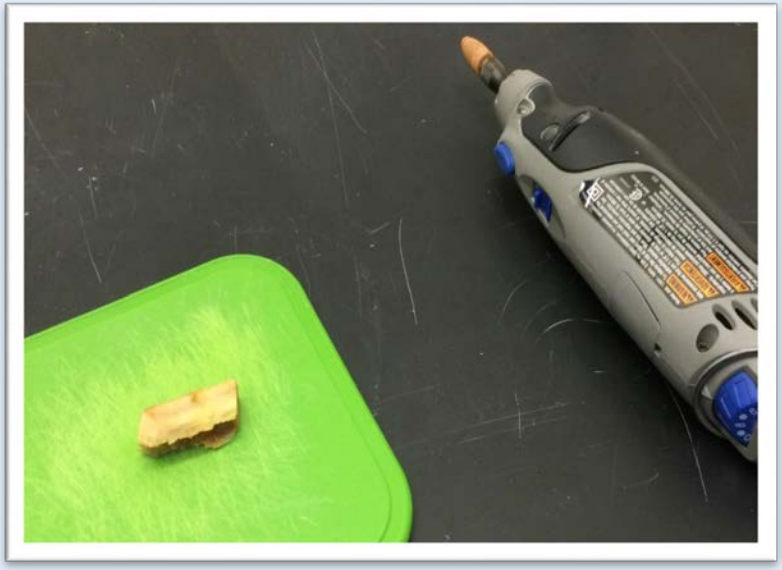
Bones & Teeth

- Humerus
- Femur
- 3rd Distal Hand Phalanx
- 3rd Distal Foot Phalanx
- Canine
- Incisor
- Bone Marrow

Hard Tissue Preparation

Bone: cleaned, dried, sanded & cut

Teeth & bone pieces: washed, dried & pulverized



Soft Tissue Preparation

Samples Collected

Samples cut into (20mg)

Heart

Extraction

Bone & Teeth Samples (~100mg powder) digested with 360 uL Buffer ATL & 20 uL proteinase K overnight

Hair & Nails

Samples digested with 300 uL Buffer ATL, 20 uL proteinase K & 20 uL 1M DTT overnight

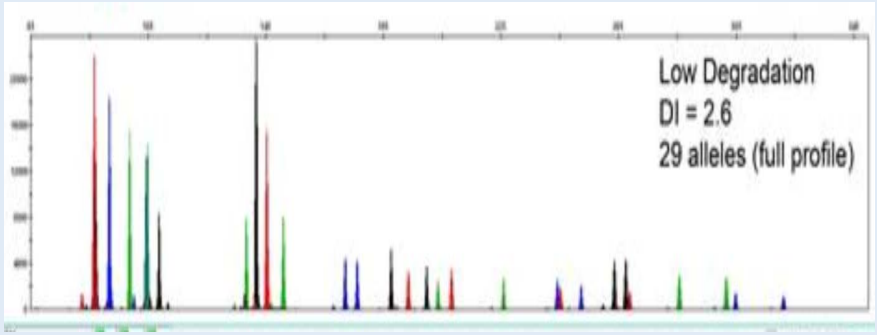
Soft tissue

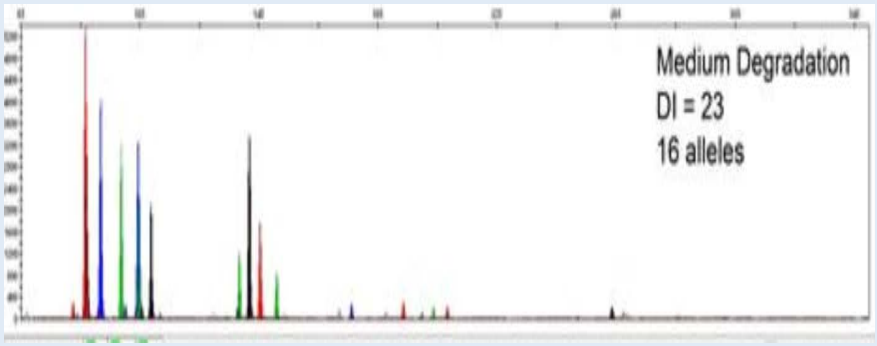
samples did not have an extra digestion step

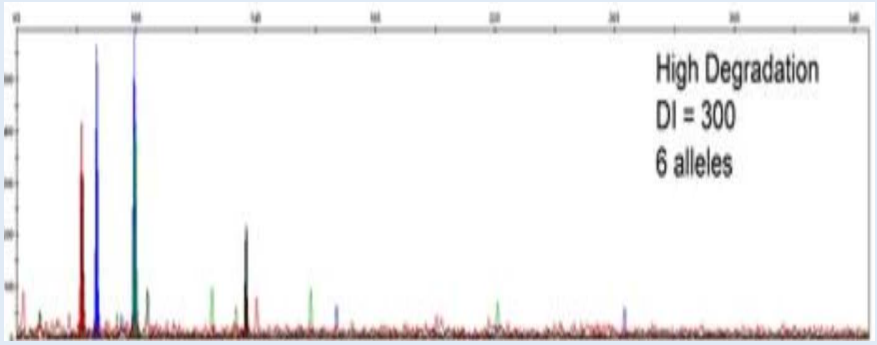
QIAamp® FFPE Tissue Kit

DNA Quantification

- Quantifiler® Trio DNA Quantification Kit
  - 7500 Real-Time Thermal Cycler
- Total Human and Male DNA quantity
- IPC (inhibition)
- DNA Degradation (Degradation Index)
  - Ratio of the small amplicon quantity (80bp) to large amplicon quantity (214bp)
  - The larger the DI value, the more degraded the sample is



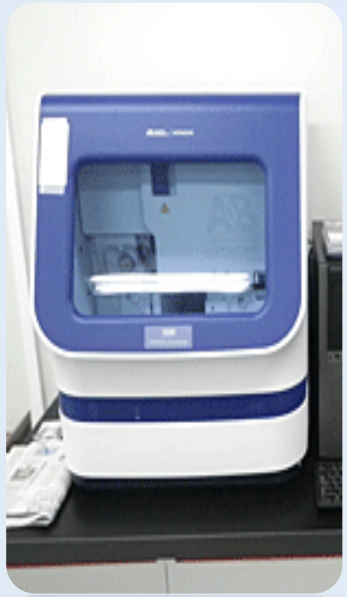




STR Analysis

- GlobalFiler® PCR Amplification Kit
  - GeneAmp® PCR System 9700

- PCR products detected via ABI Prism 3500 Genetic Analyzer
  - 36cm capillary and POP-4 Polymer
  - GeneMapper ID-X



Results & Discussion – DNA Concentration

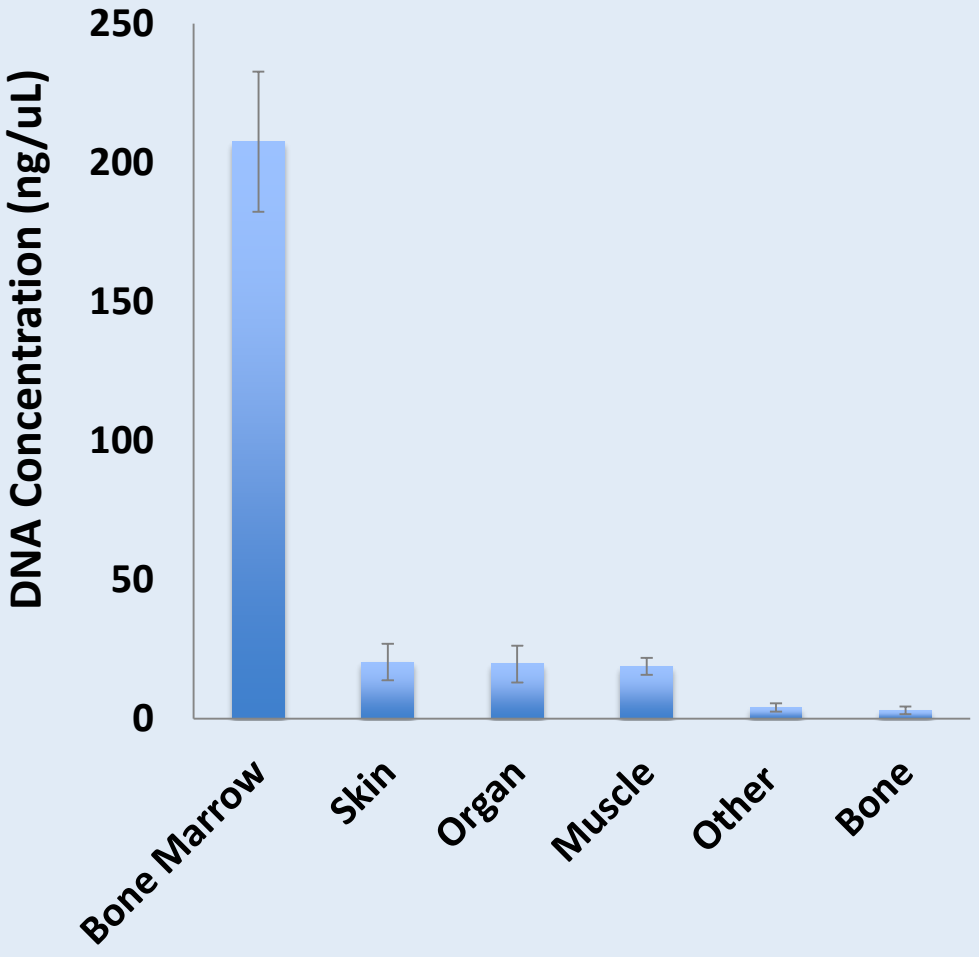
- Variation between the three cadavers

	Cadaver 1	Cadaver 2	Cadaver 3
Average [DNA] (ng/µL)	25.9	5.7	28.1
DNA Concentration (ng/µL)	**Proportion of Samples (%)**		
> 20	35	5	34
10 – 20	18	18	13
2 – 10	17	18	20
0.02 – 2	28	44	27
0.002 – 0.02	0	9	4
Below 0.002	2	6	2

Difference in yield between cadavers was highly significant ($F_{2,90} = 4.99$, $p < 0.01$).

DNA Concentration

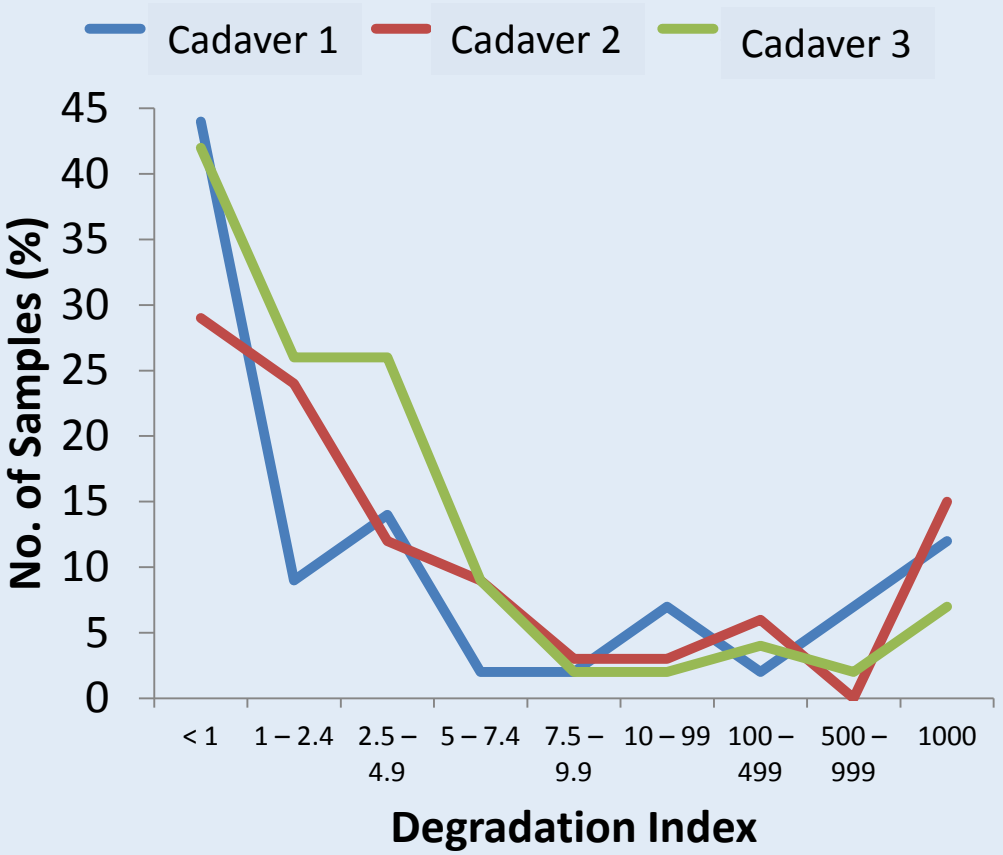
- Overall, bone marrow resulted in the highest yields of all samples
- Skin, organ and muscle similar
- Other (cartilage, clippings, hair, teeth & tendon) and bone were similar
- Nails, skin, stomach, hair and teeth consistently yielded the lowest amounts of DNA.



Difference in DNA Concentration between tissue types was significant ($F_{4,94} = 3.18$, $p < 0.05$)

Results & Discussion – DNA Degradation

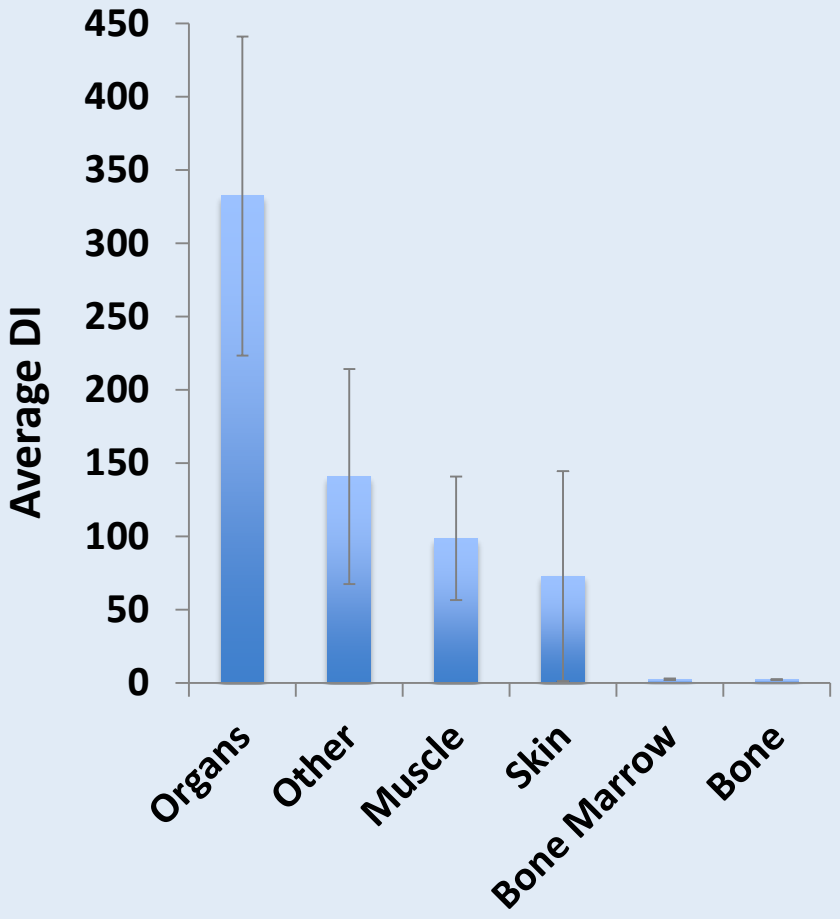
- 9 out of 122 samples were too degraded to determine a DI value (the large amplicon could not be amplified)
- Levels of DI were consistent across the three cadavers



DI values were not found to differ significantly between the cadavers ($F_{2,96} = 0.69$, $p = 0.5$).

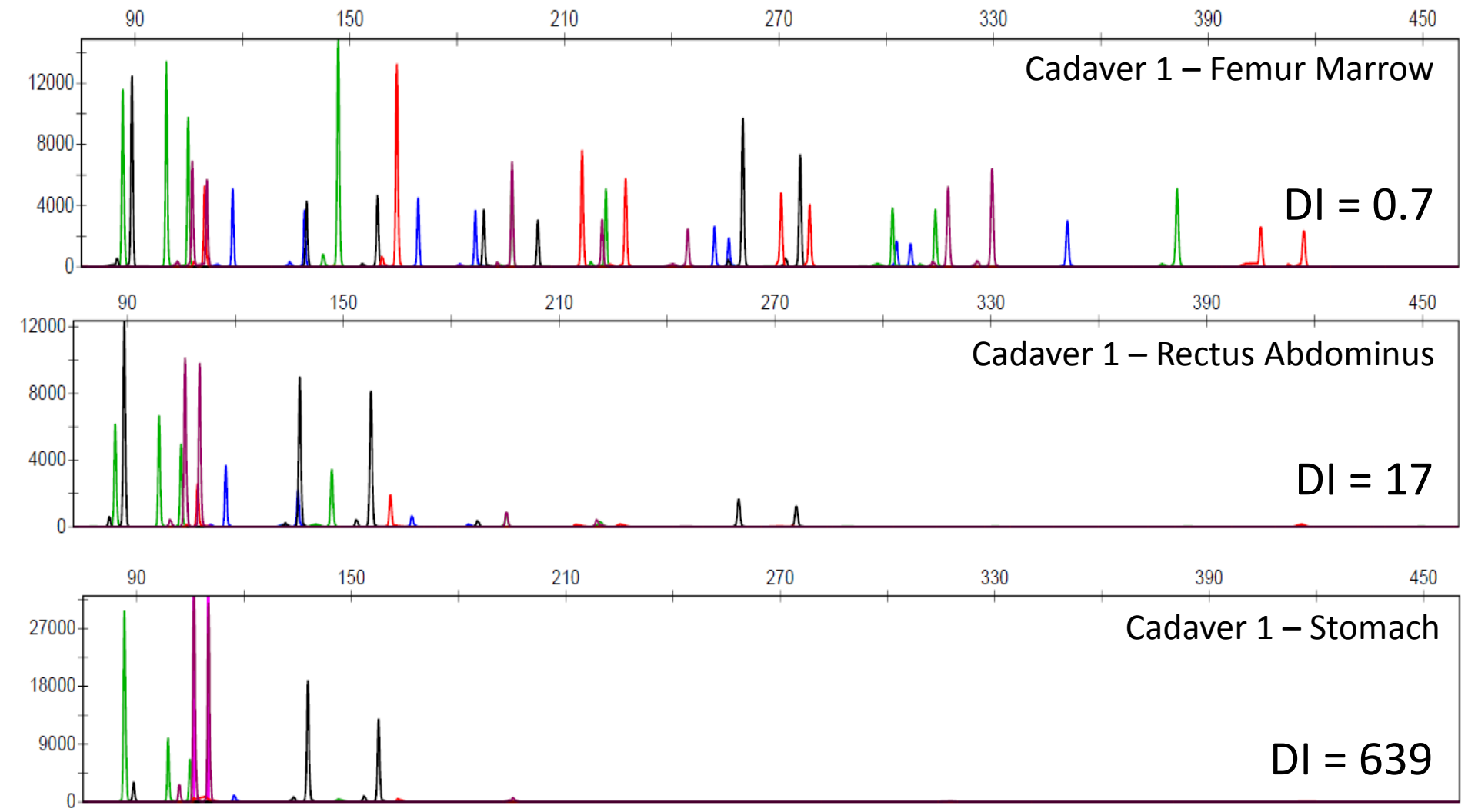
DNA Degradation

- Variation was seen across the various tissue types
- Organs were the most damaged; bone and bone marrow the least
- Consistent with the hypothesis that highly perfused tissues are more highly damaged



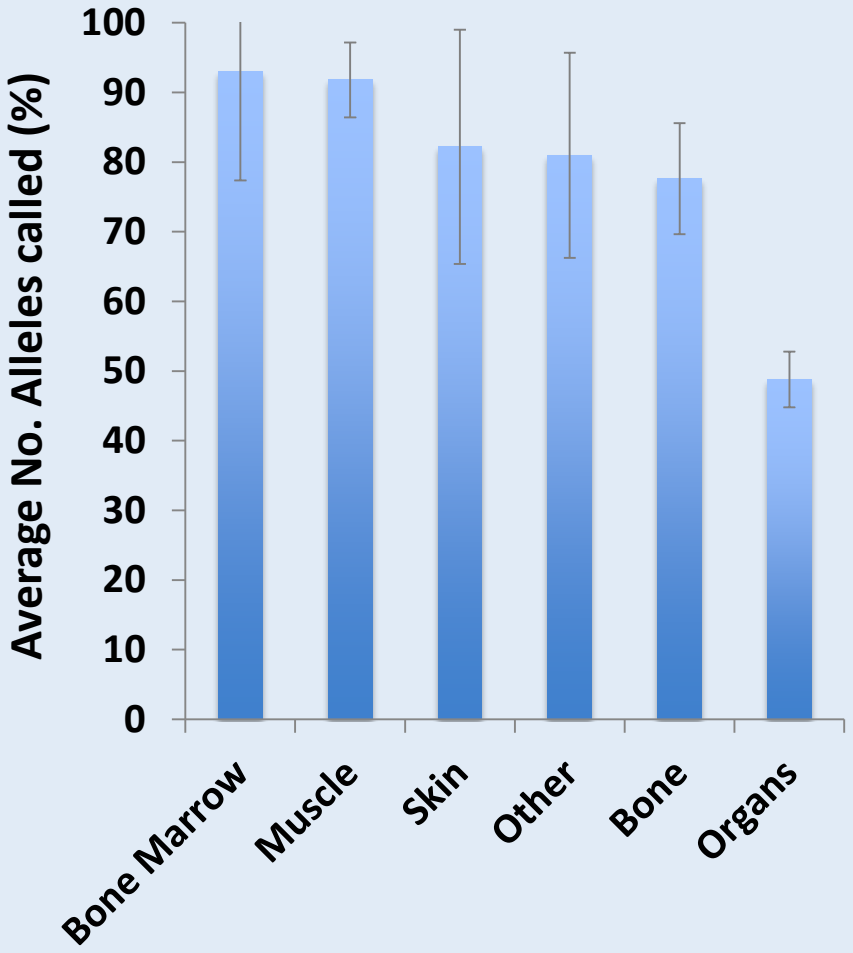
The DI values were found to differ significantly based on tissue type ($F_{4,94} = 3.25$, $p < 0.05$).

DNA Degradation

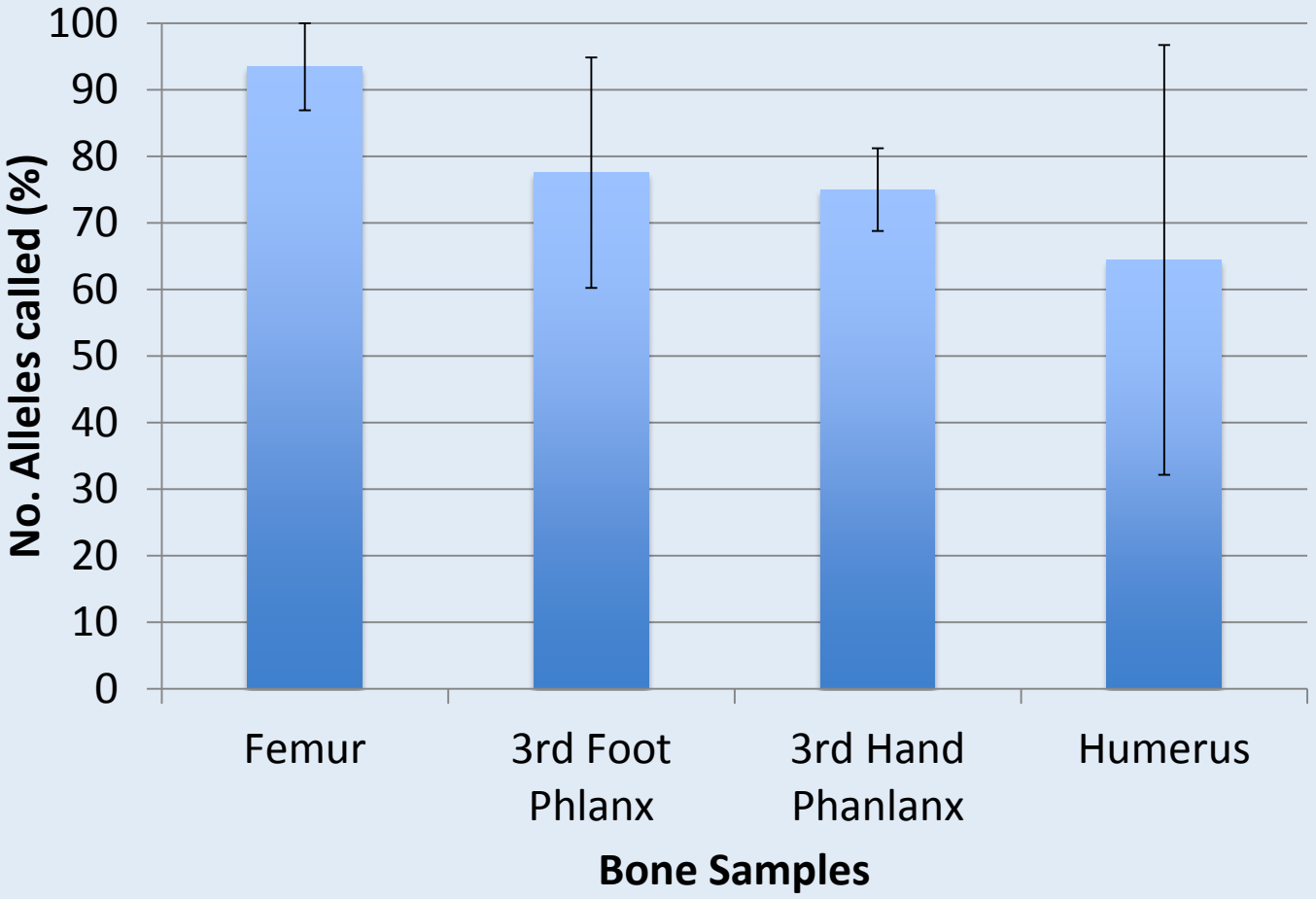


Results & Discussion – STR Analysis

- STR success was found to be dependent on the donor ($F_{2,87} = 5.81$, $p < 0.01$).
- Bone marrow and muscle tissue types generated on average the most complete STR profiles
- Internal organs consistently yielded the least complete
- 9 samples consistently produced full profiles
  - Muscle – Flexor Digitorum brevis, Gastrocnemius, Rectus Femoris & Thenar
  - Fingernails, calcaneal & patella tendon



STR Analysis



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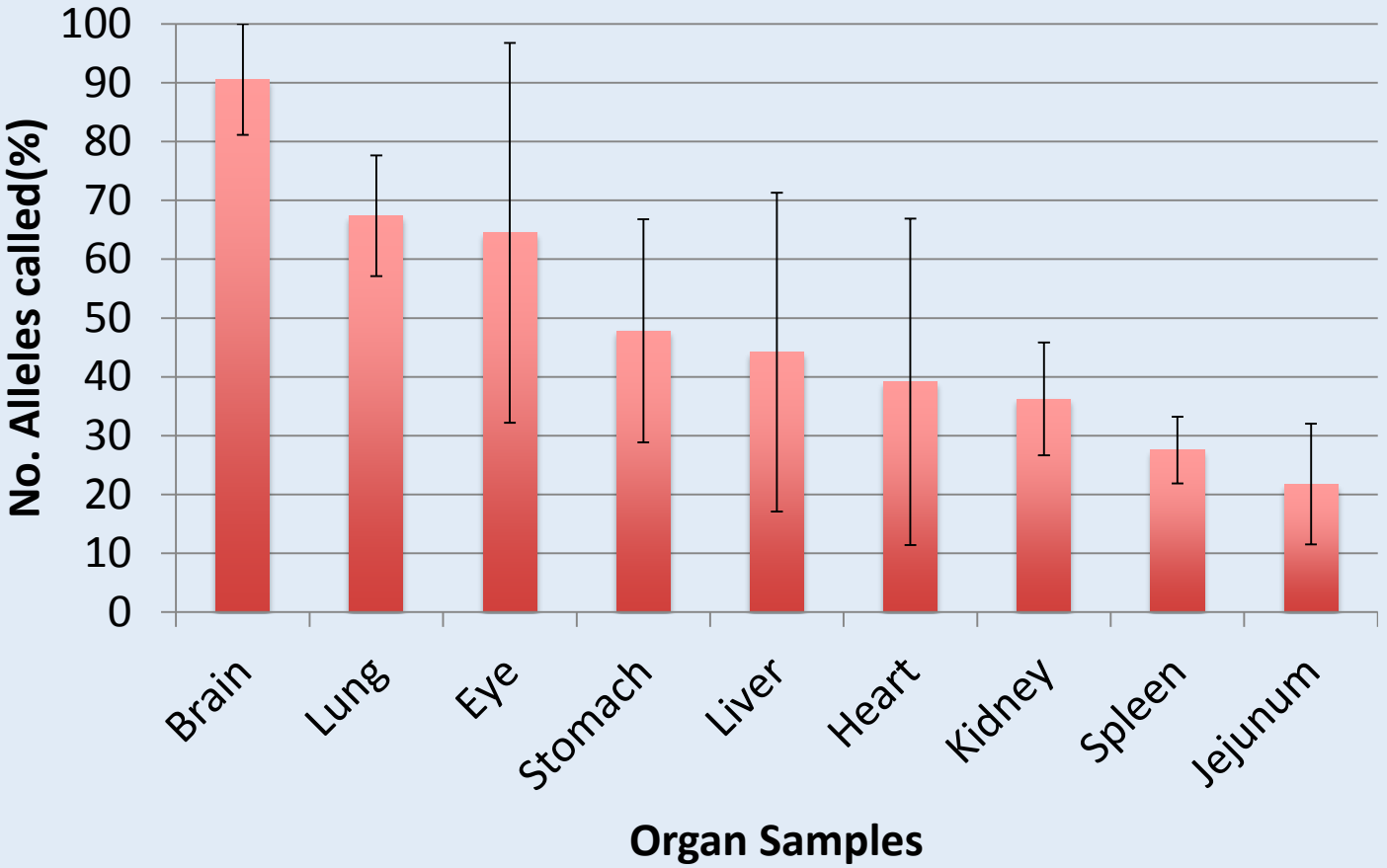

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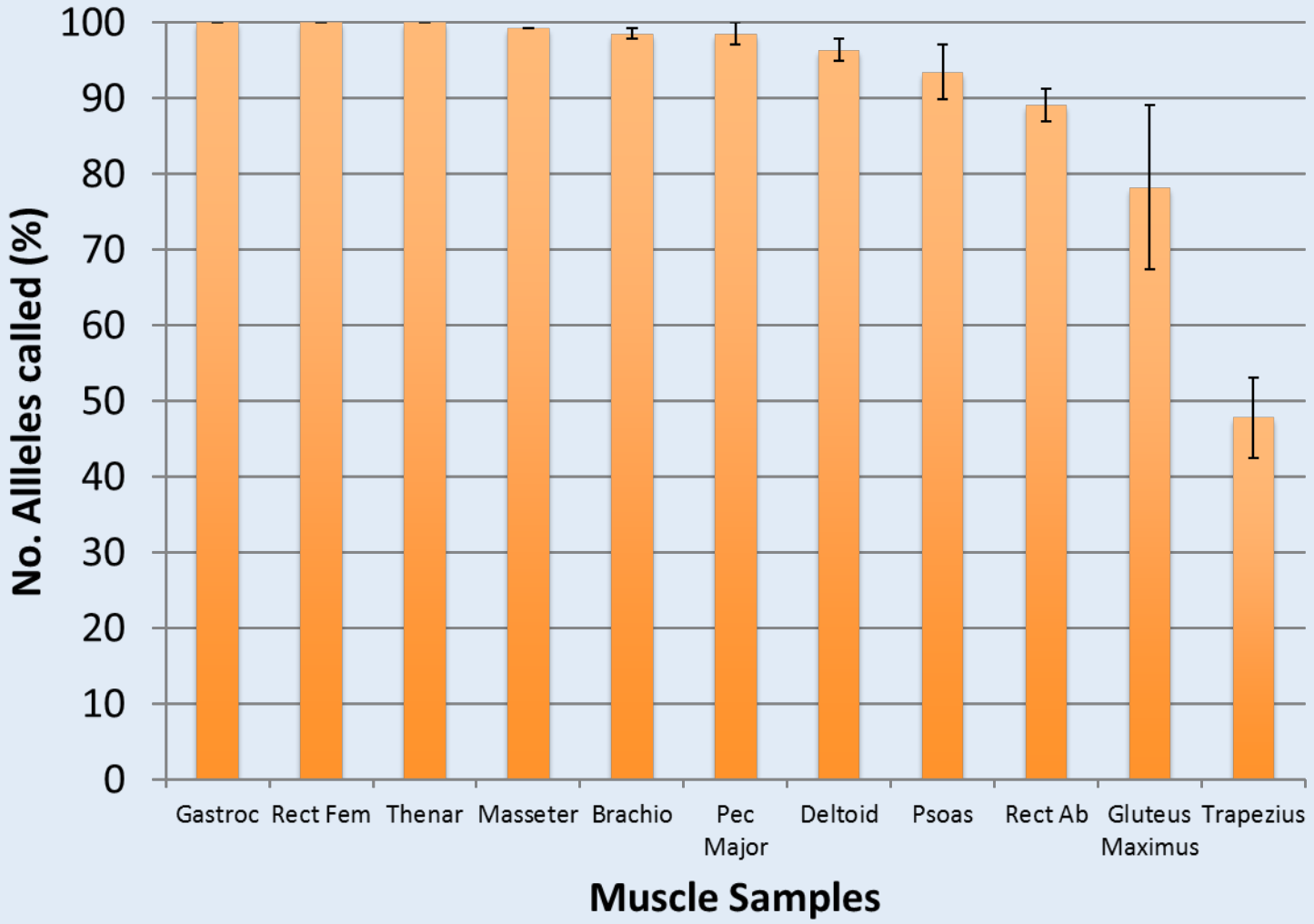
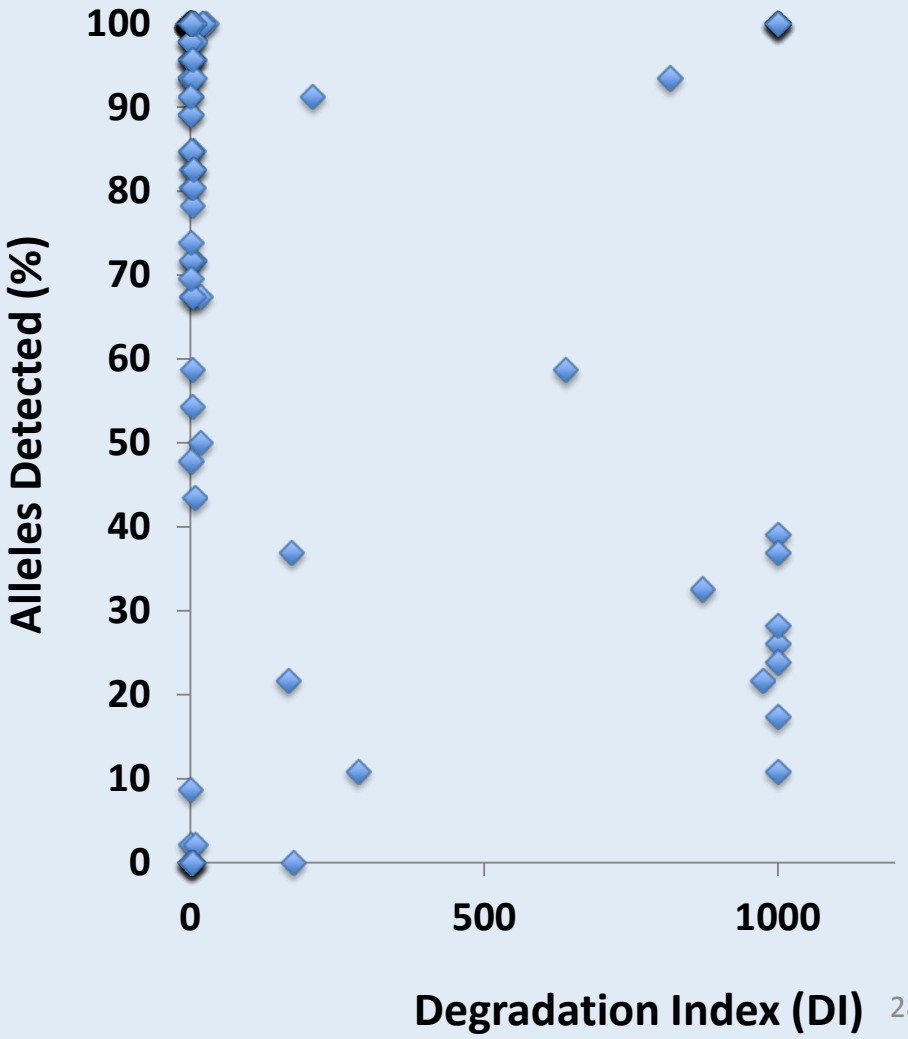


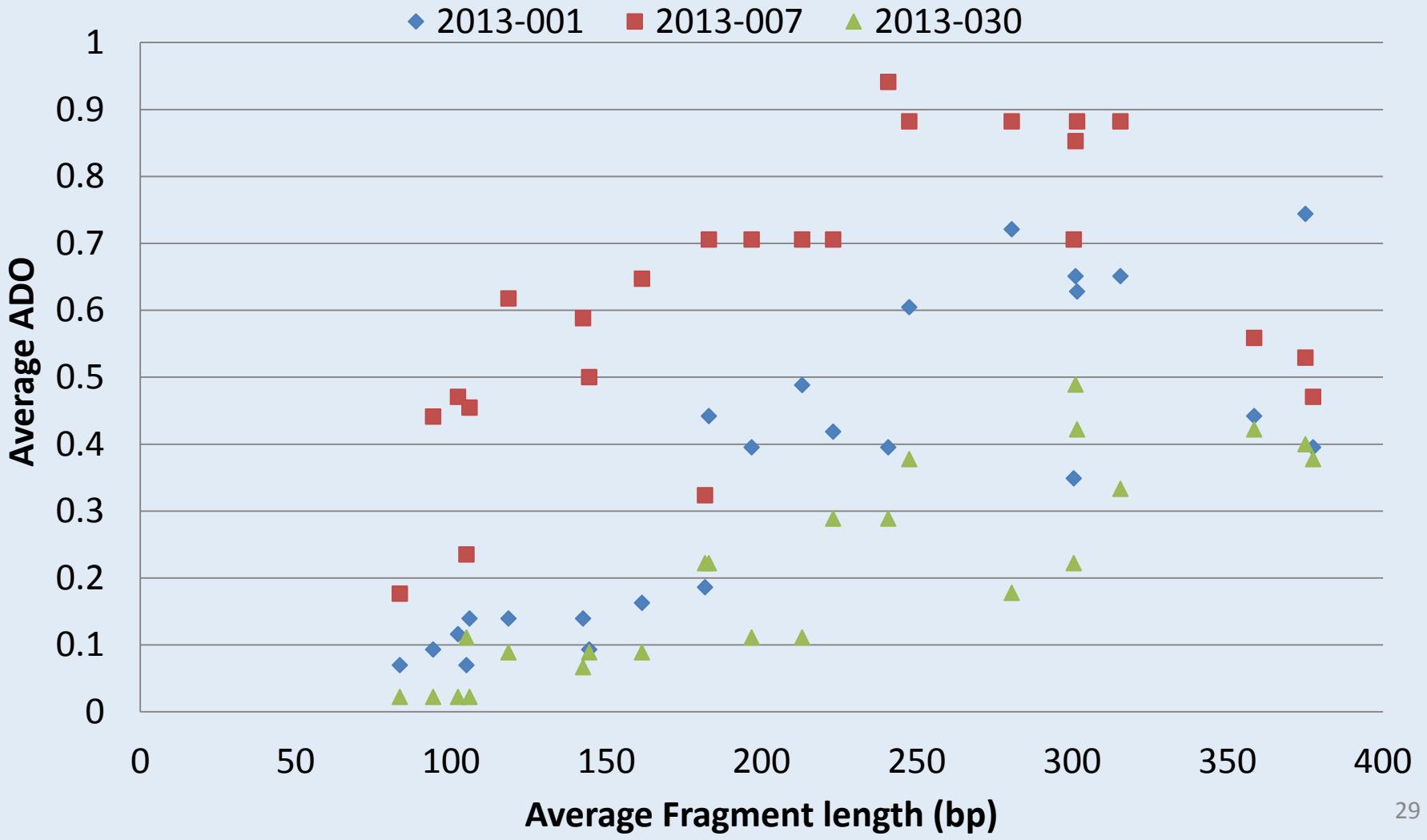
DI vs STR Success

- Data suggest that DI values are not predictive of STR success with FD samples

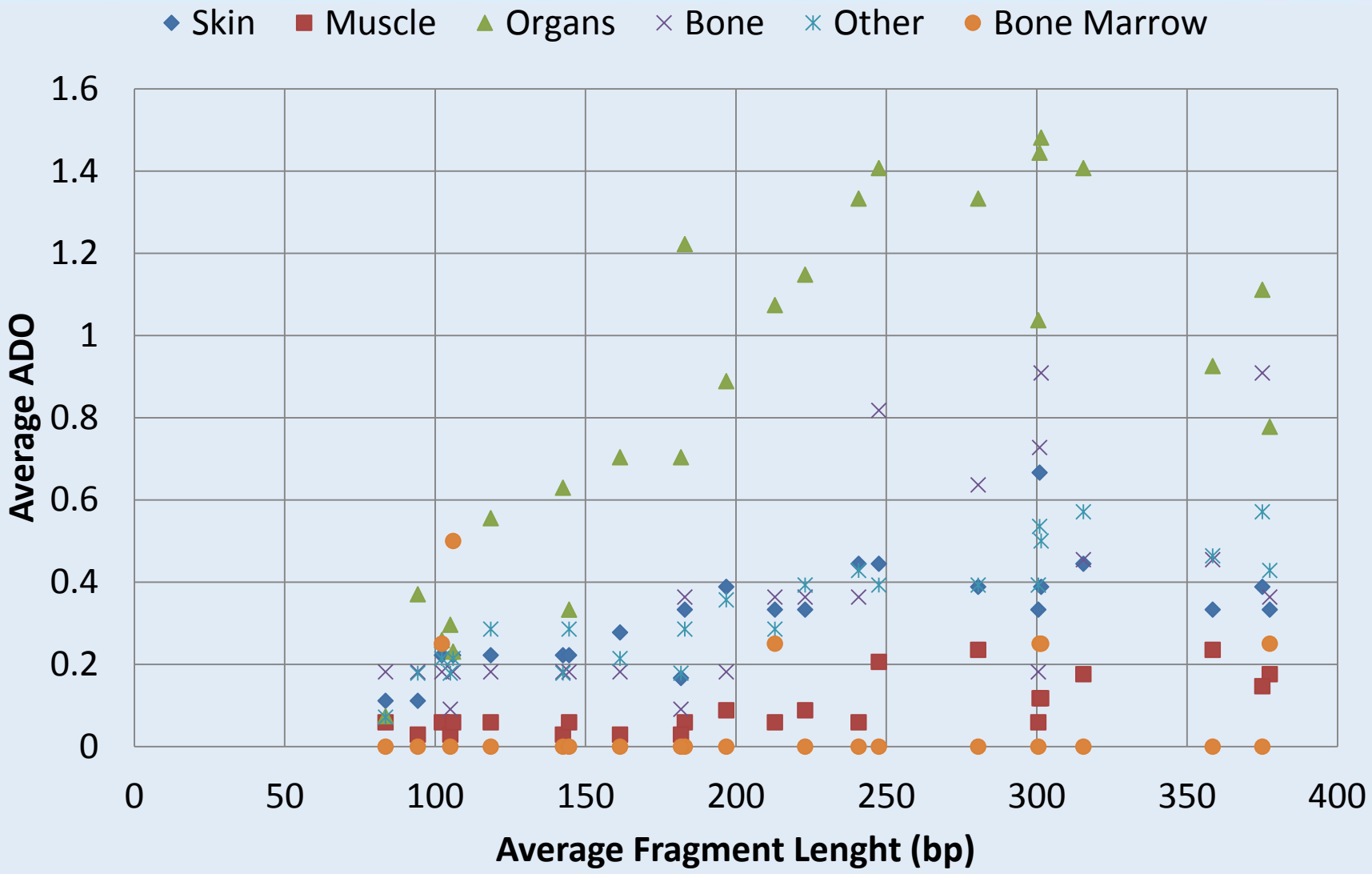
- Samples with low DI values and partial/no profiles and samples with extremely high DI values with partial/full profiles



Allele Drop Out vs Amplicon Size



Allele Drop Out vs Amplicon Size



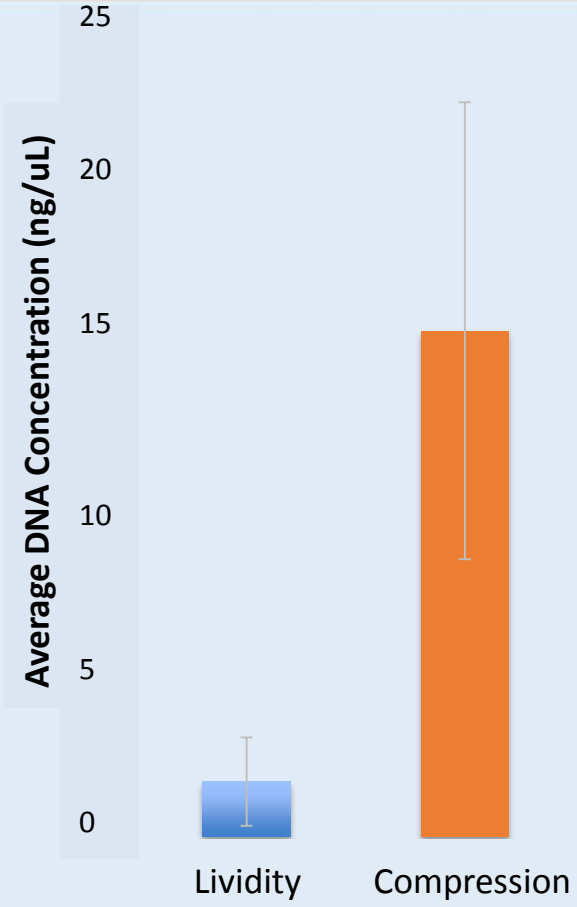
Results and Discussion – Livor Mortis

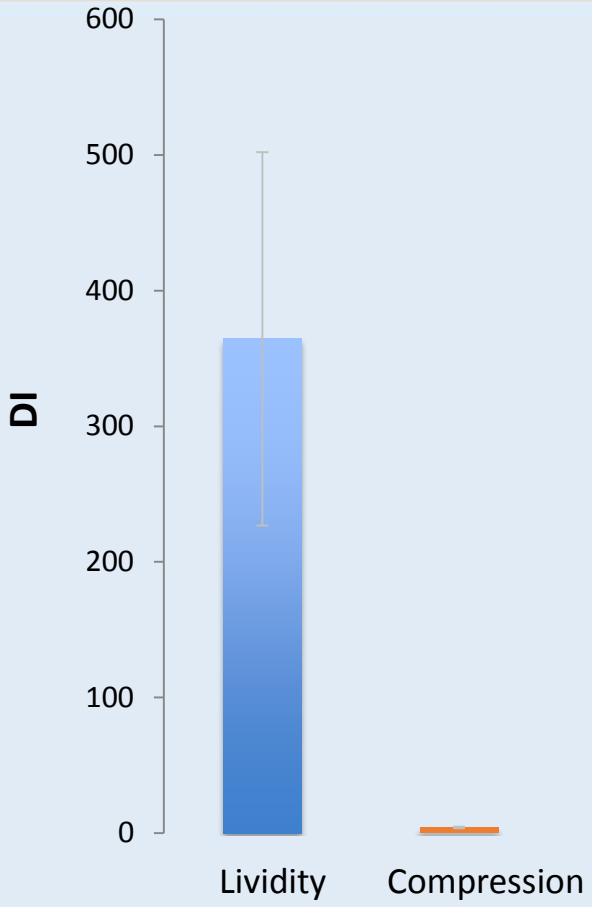
- Skin, fat & muscle sampled from two areas:
  - Trapezius - livor mortis, blood pooling (reddish)
  - Gluteus Maximus – compressed (white)

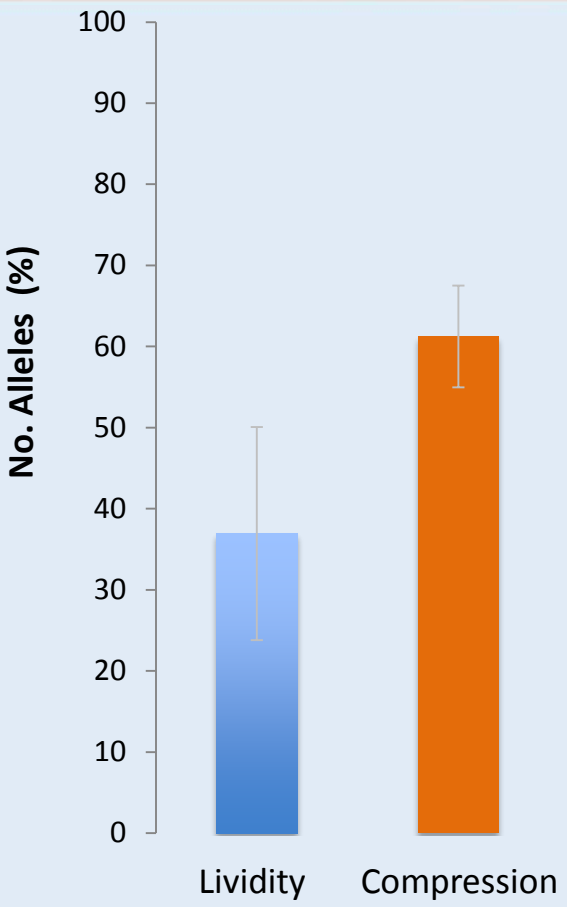
- Data suggest that areas under compression may have less damaged DNA than areas with blood pooling

Not the actual cadaver used

Livor Mortis Cont.







No statistical difference in [DNA] ($p = 0.12$)

Statistical difference in DI ($p < 0.5$)

Statistical difference in success ($p < 0.5$)

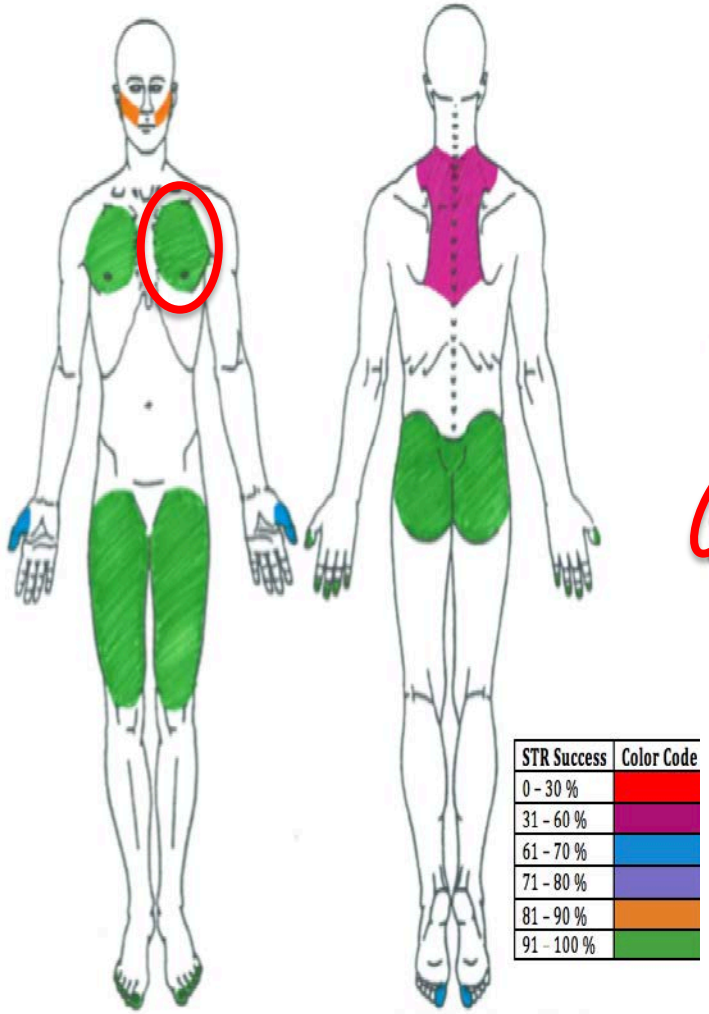
Conclusions

- Guidance may be provided to the forensic community on which tissues from embalmed human remains will most likely generate more complete STR profiles.

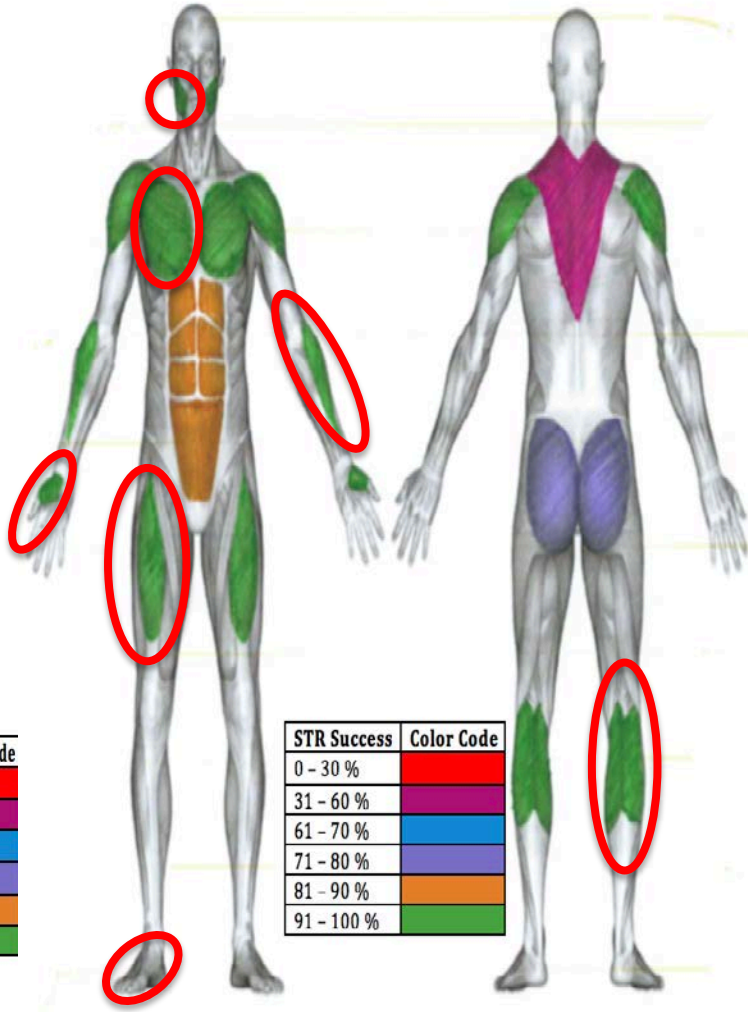
- While bone samples did result in both partial to full profiles, skin and muscle samples resulted in higher average success rates.
  - These samples are also much easier to obtain and extract DNA from than bone and teeth.

STR Success

Skin & Tendon



Muscle



Bone

Acknowledgements

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  - Natalia Czado, Samantha Tippen
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- Meredith Turnbough for her valuable advice
- Sheri Olsen (Life Technologies) for kindly providing the QuantiFiler® Trio DNA Quantification kit used in this study

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References

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2. Mundorff A, Davoren J (2014) Examination of DNA Yield Rates for Different Skeletal Elements at Increasing Post Mortem Invtervals. Forensic Sci Int Genet 8:55-63.
3. Vernarecci S, et al (2015) Quantifiler Trio Kit and Forensic Samples Management: A Matter of Degradation. Forensic Sci Int Genet 16:77-85.

For more information please see *Comparison of DNA Yield and STR Success Rates from Different Tissues in Embalmed Bodies* [submitted for review in the Journal of Legal Medicine]

Questions?

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