Rapid DNA Identification of Human Remains in Diverse Conditions: What Works, What Doesn't

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EMERGING FORENSIC SCIENTIST AWARD

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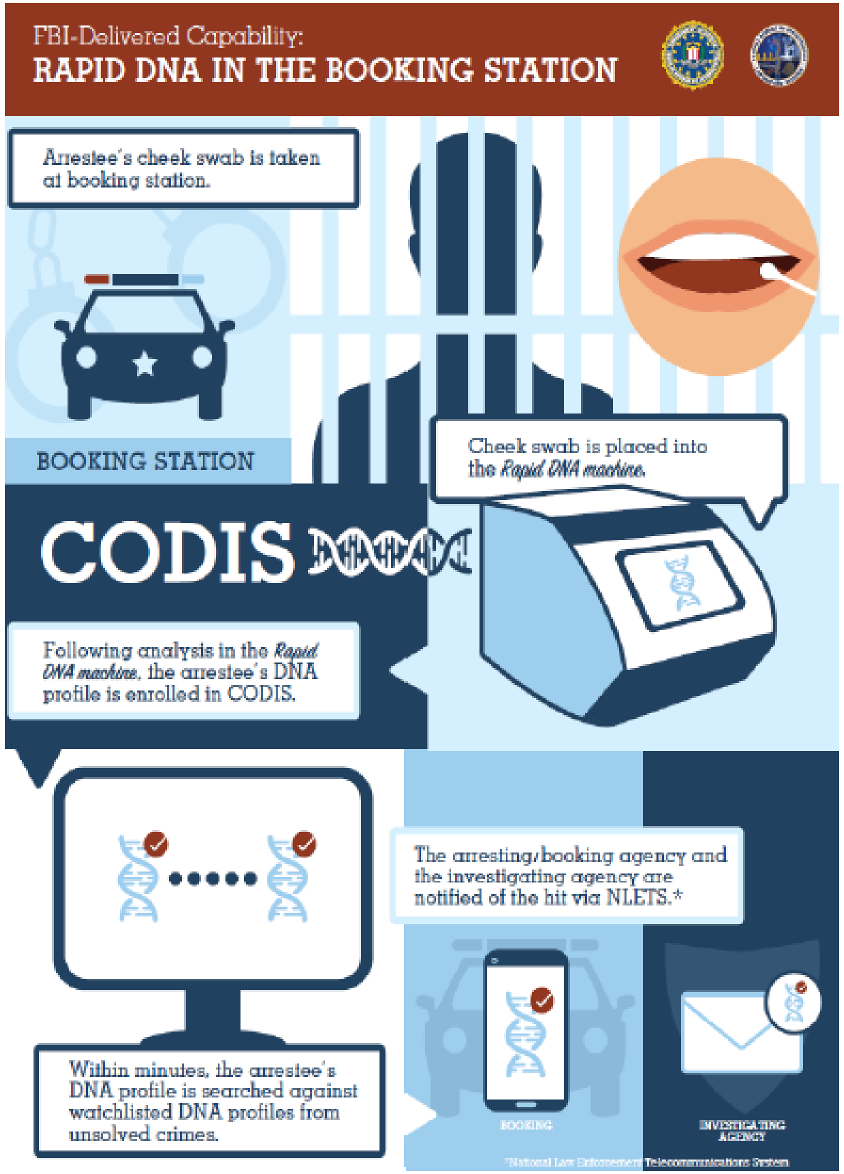
Rapid DNA

- FBI defines Rapid DNA "*a fully automated process of developing a DNA profile from a mouth swab. This happens in one to two hours – without the need for a DNA laboratory or DNA intervention and review*"[1]
- Currently used for booking stations to submit arrestee's DNA profile to CODIS[1]
- July 1, 2025, FBI QAS standards allow for the use of Rapid DNA in forensic casework[1]



FBI-Delivered Capability:
RAPID DNA IN THE BOOKING STATION

Arrestee's cheek swab is taken at booking station.

Cheek swab is placed into the *Rapid DNA machine*.

Following analysis in the *Rapid DNA machine*, the arrestee's DNA profile is enrolled in CODIS.

Within minutes, the arrestee's DNA profile is searched against watchlisted DNA profiles from unsolved crimes.

The arresting/booking agency and the investigating agency are notified of the hit via NLETS.*

*National Law Enforcement Telecommunications System

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[1] https://le.fbi.gov/science-and-lab/biometrics-and-fingerprints/codis/rapid-dna#:~:text=Rapid%20DNA%2C%20or%20Rapid%20DNA,any%20human%20intervention%20and%20review.

Rapid DNA Applications

- Investigative Leads Testing
- Booking Stations
- Casework Reference Samples and Hit Confirmations
- **Disaster Victim Identification (DVI)**

Rapid DNA instruments

RapidHIT ID System

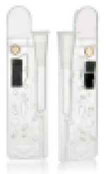
- Thermo Fisher Scientific
- 1 sample 90 min
- 2 types of cartridges

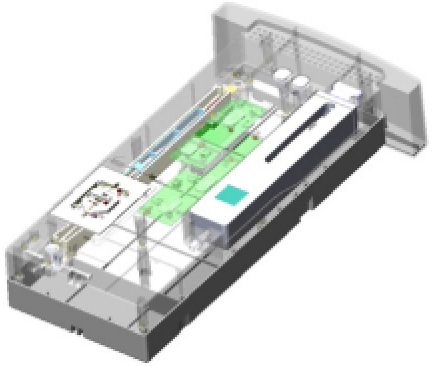
ANDE 6C Instrument

- ANDE
- 5 samples
- Specialized swab and chips

RapidHIT ID System

Lysis and amplification are performed onboard the sample cartridge
The primary cartridge encapsulates capillary electrophoresis and detection steps





Step		Cartridge
1	**Lyse sample**	**Sample Cartridge**
2	**Amplify product**	**Sample Cartridge**
3	**Transfer STR/ILS to electrophoresis**	
4	**CE and detect**	**Primary Cartridge**
5	**Analyze and upload results**	

Thermo Fisher Scientific Human Identification | January 2024

RapidHIT ID System Cartridges

ACE GlobalFiler Express

- Reference Samples
- Booking Station
- 500 µL lysis buffer
- **28** PCR cycles
- 1 filter paper punch

RapidINTEL™ Plus

- **Moderate to high-yield** crime scene samples
- Investigative Leads
- 500 µL (general) or 100 µL (specialized) protocols
- **30** PCR cycles
- Internal Quality Control (IQC) markers
- Quantification markers
- 1 oblong filer punch

Research Objective

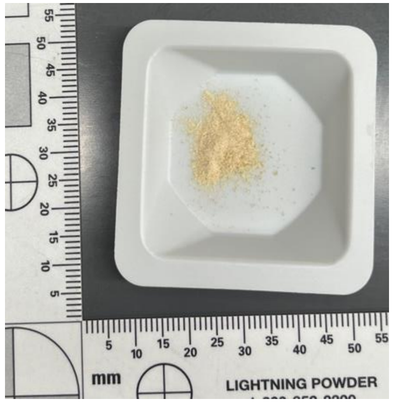
- Identify the best way to prepare bones by testing different degrees of “fineness” for the RapidHIT ID instrument by using an off-instrument mock rapid CE method
- Explore if pretreatment methods would improve results for bones
- Evaluate cadaveric samples taken at different periods of decomposition to assess when rapid would be the most advantageous.

Workflow

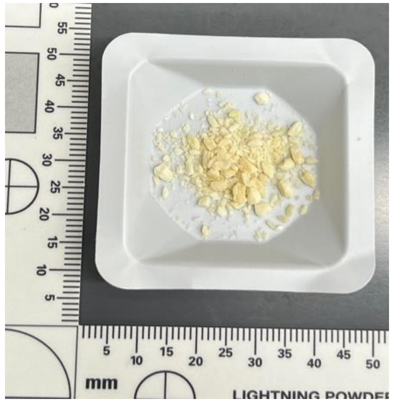
Bone Selection
Good DNA Yield (10ng/ul)
Low DNA Yield (0.0122 ng/ul)

Quant based on Quantifiler Trio 50mg

Powder

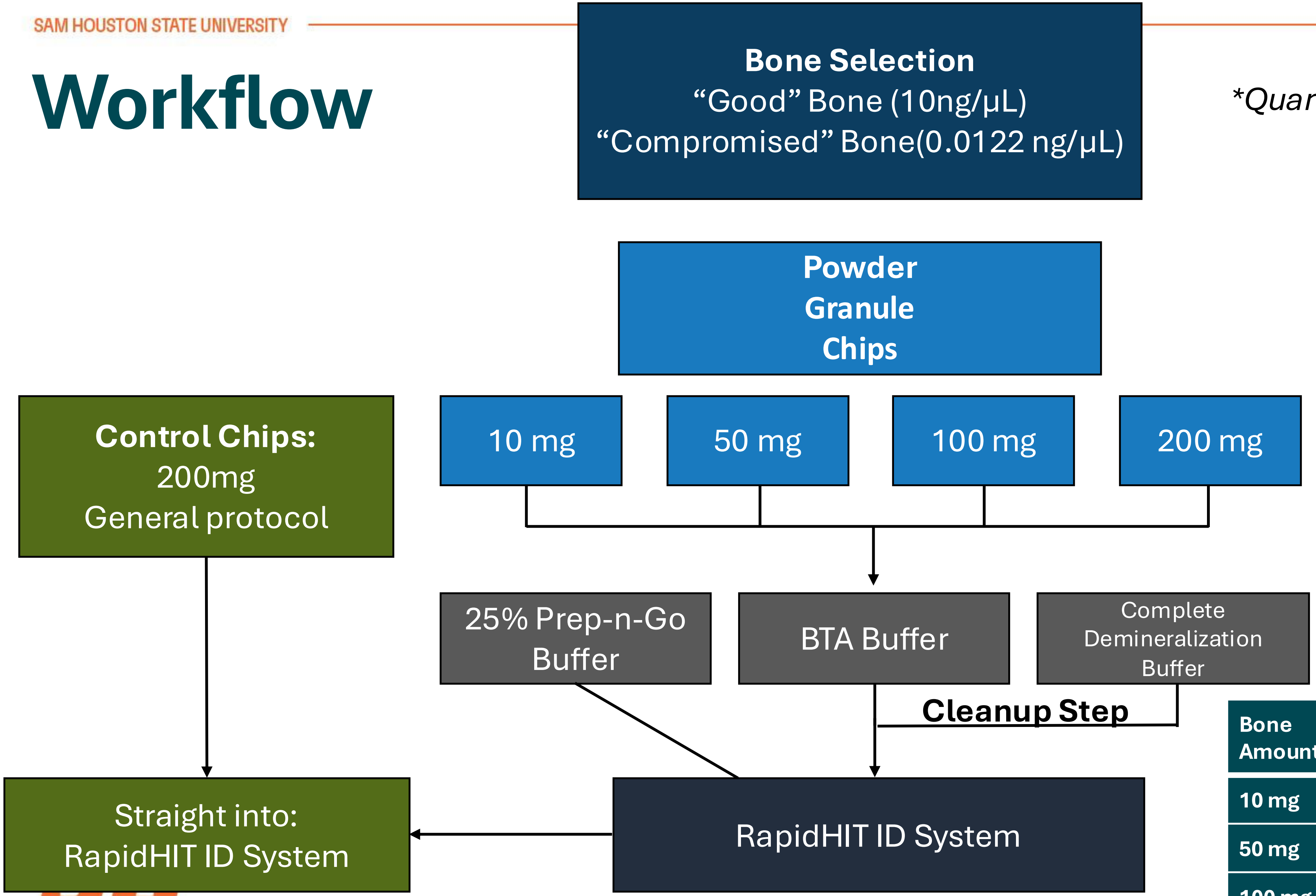


Spice Grinder



Chips

Workflow



Bone Selection
“Good” Bone (10ng/µL)
“Compromised” Bone(0.0122 ng/µL)

Quant based on Trio 50mg

Powder
Granule
Chips

Control Chips:
200mg
General protocol

10 mg

50 mg

100 mg

200 mg

Bone Amounts

25% Prep-n-Go Buffer

BTA Buffer

Complete Demineralization Buffer

Lysis Amounts

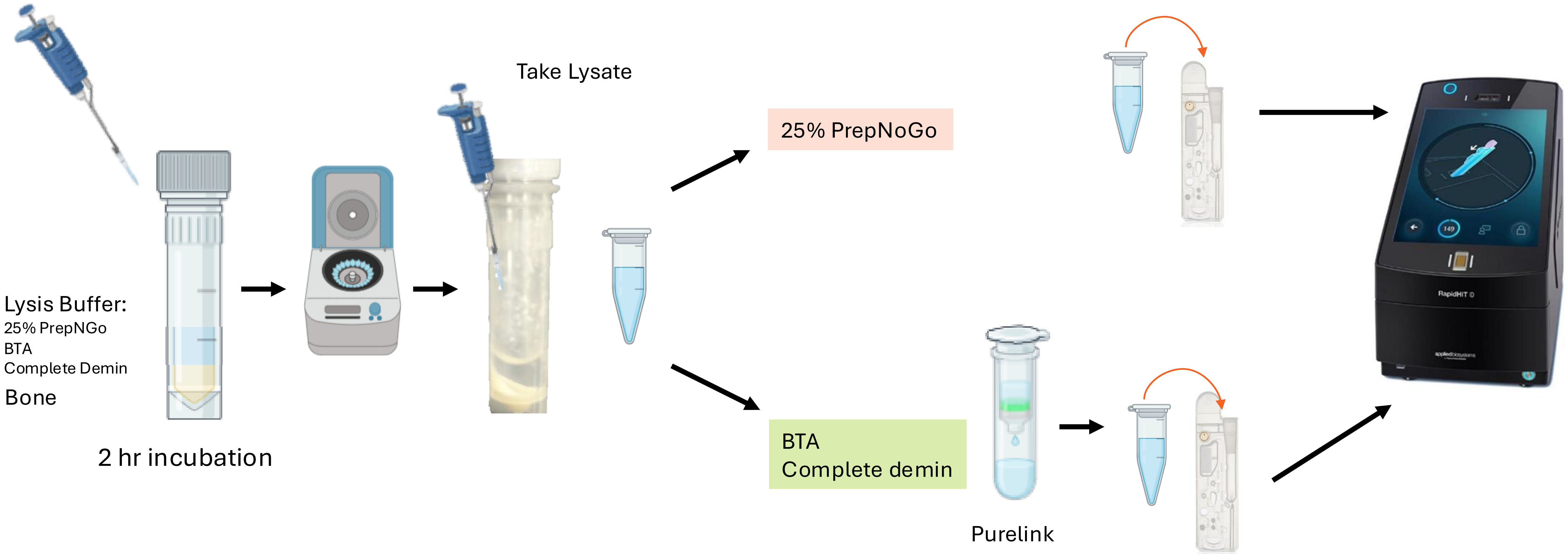
Cleanup Step

Straight into:
RapidHIT ID System

RapidHIT ID System

Bone Amount	Powder (µL)	Spice Grinder (µL)	Chips (µL)
10 mg	100	100	N/A
50 mg	200	200	200
100 mg	400	300	300
200 mg	600	400	400

Rapid Workflow



Lysis Buffer:
25% PrepNGo
BTA
Complete Demin

Bone

2 hr incubation

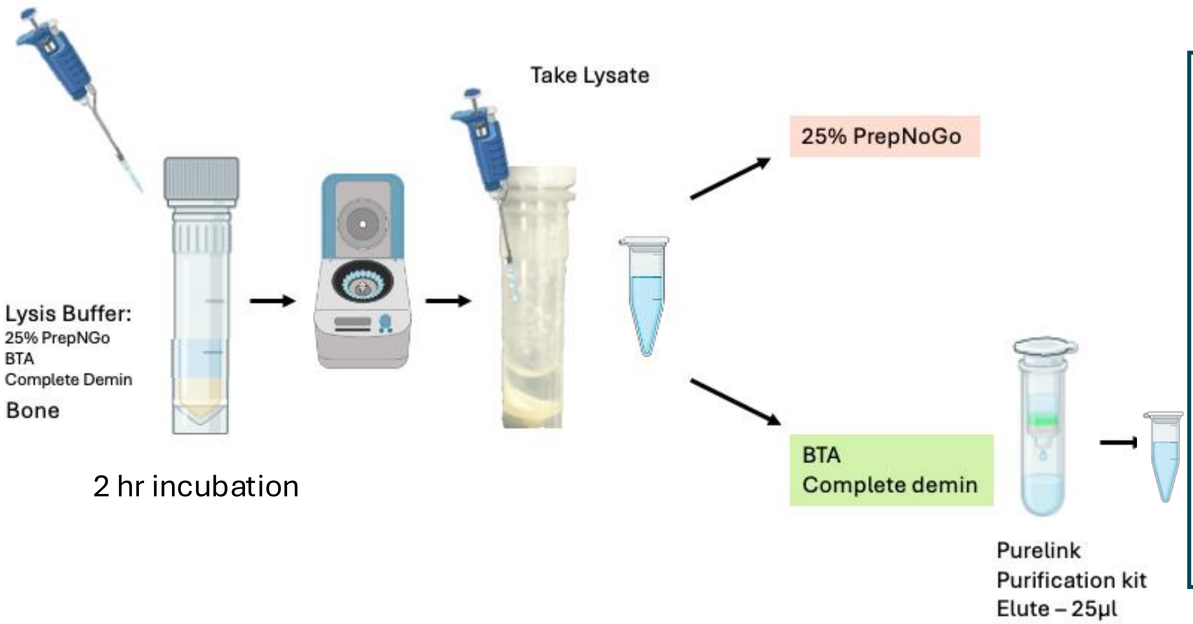
Take Lysate

25% PrepNoGo

BTA
Complete demin

Purelink
Purification kit
Elute – 25µl

Mock Rapid Workflow



2 hr incubation

RapidHIT ID System Parameters
Mock Rapid Method

Simulate Rapid Parameters:
Add 100µl
(Specialized Protocol)
85^{0}C for 12min 900 RPM

2µl for Quant
Quantifiler Trio

5µl for STR
GlobalFiler Express

Comparison of Bone Consistencies

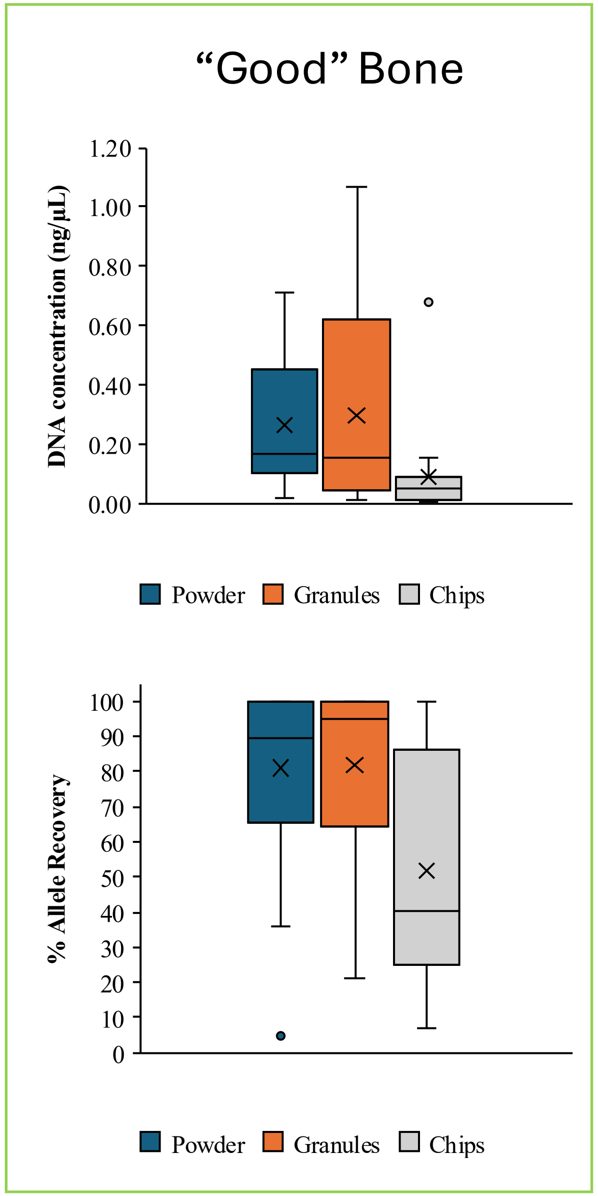
“Good” Bone

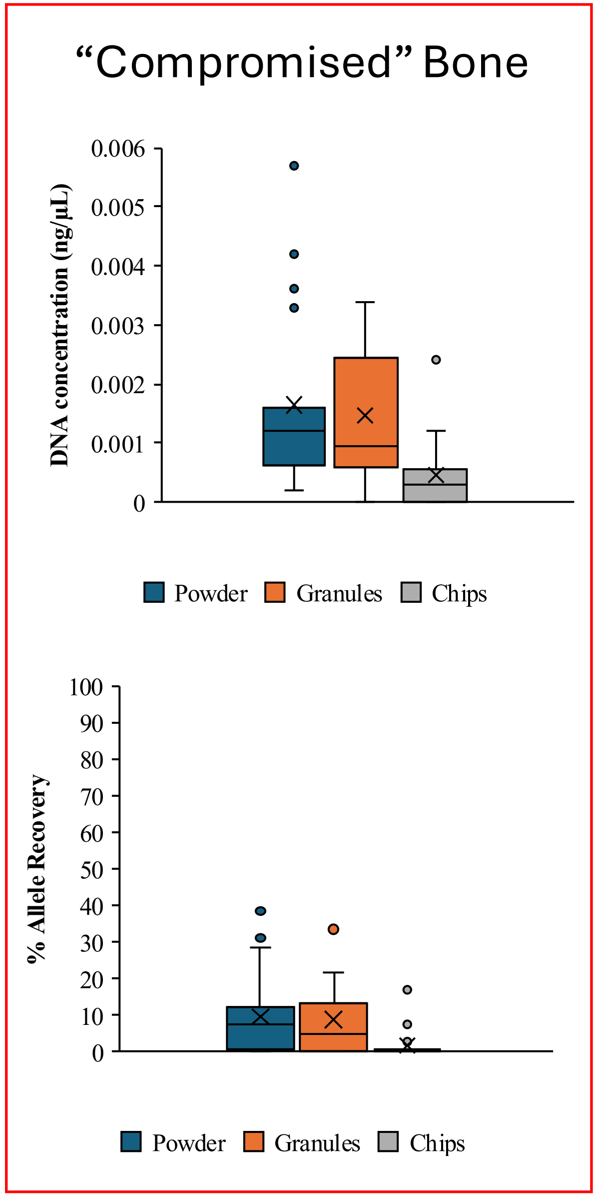
- Bone consistency had a significant effect on DNA concentration ($p < 0.05$)

“Compromised” Bones

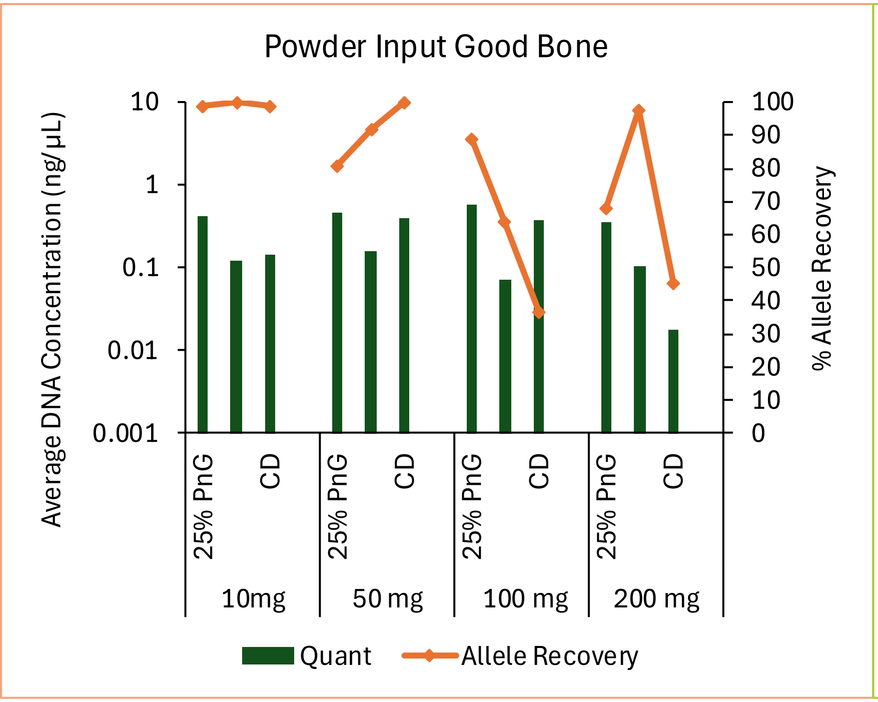
- Low quantities and partial STR profiles

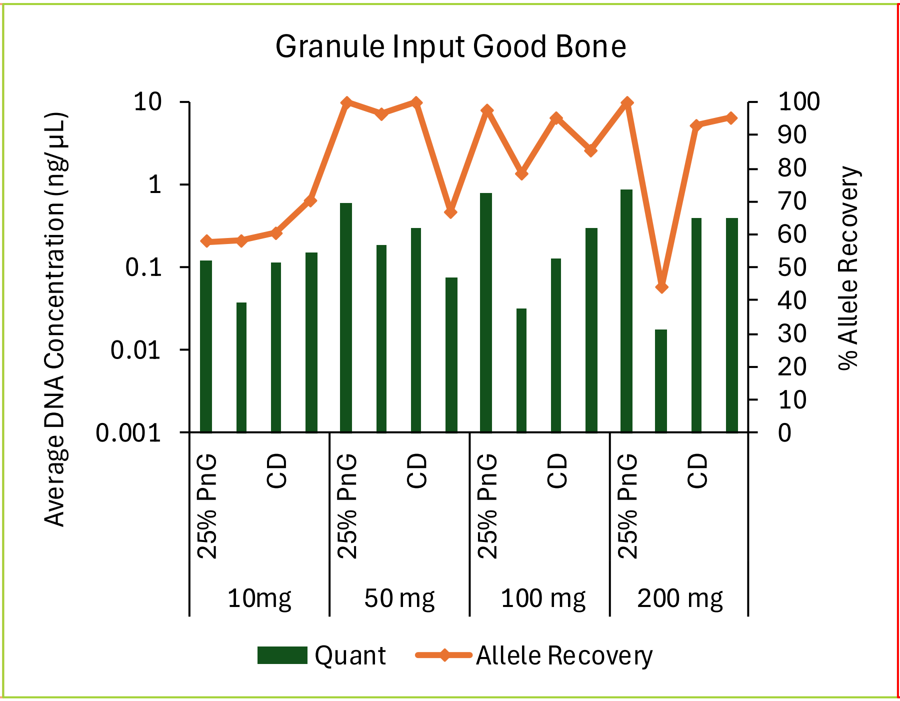
- Chips were the least successful

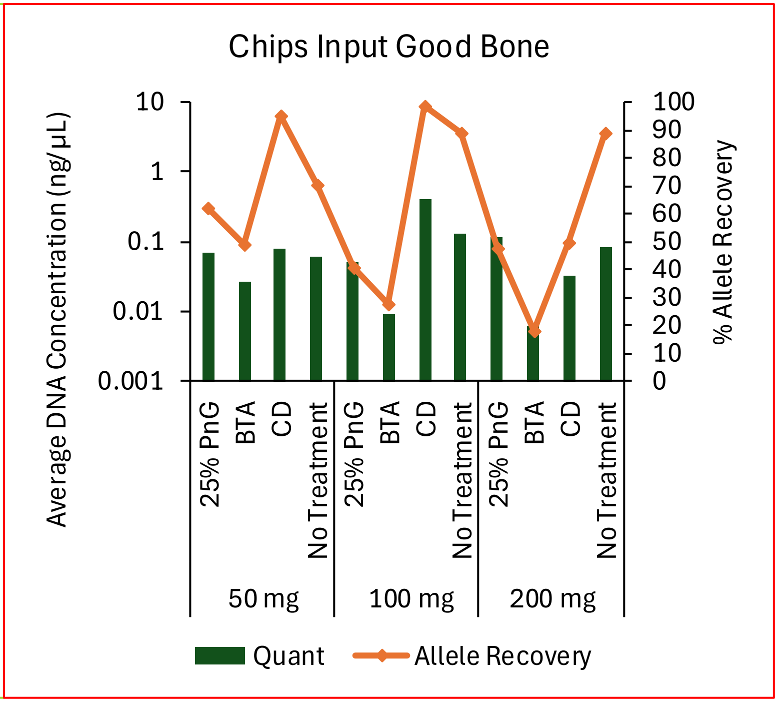

“Good” Bone


“Compromised” Bone

Bone Consistency Comparison







10 mg Powder performed most consistently among all pretreatments

Granules performed the most consistently with 50 mg or more input for DNA yield and STR recovery

No general trends observed

Comparison of Pretreatments

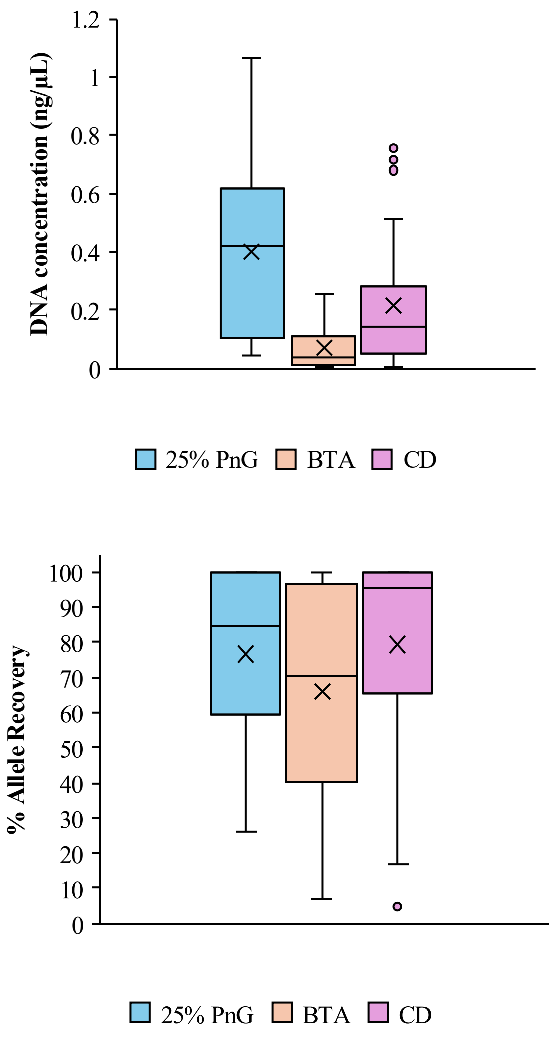
“Good” Bone

- DNA concentrations were significantly different with 25% Prep-n-Go buffer compared to other methods ($p < 0.05$)

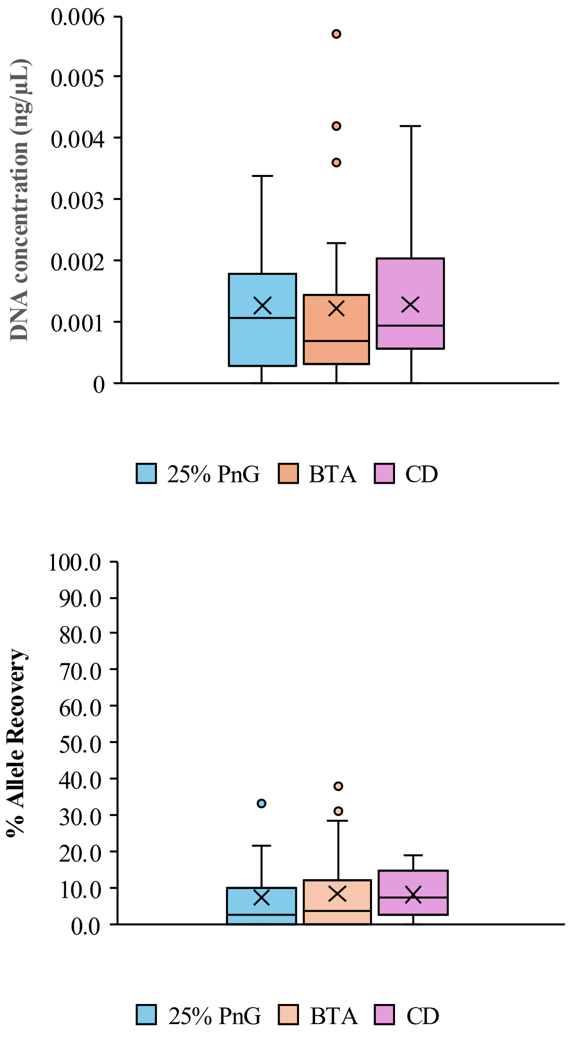
“Compromised” Bone

- No significant difference was observed between pretreatments ($p > 0.05$)

“Good” Bone



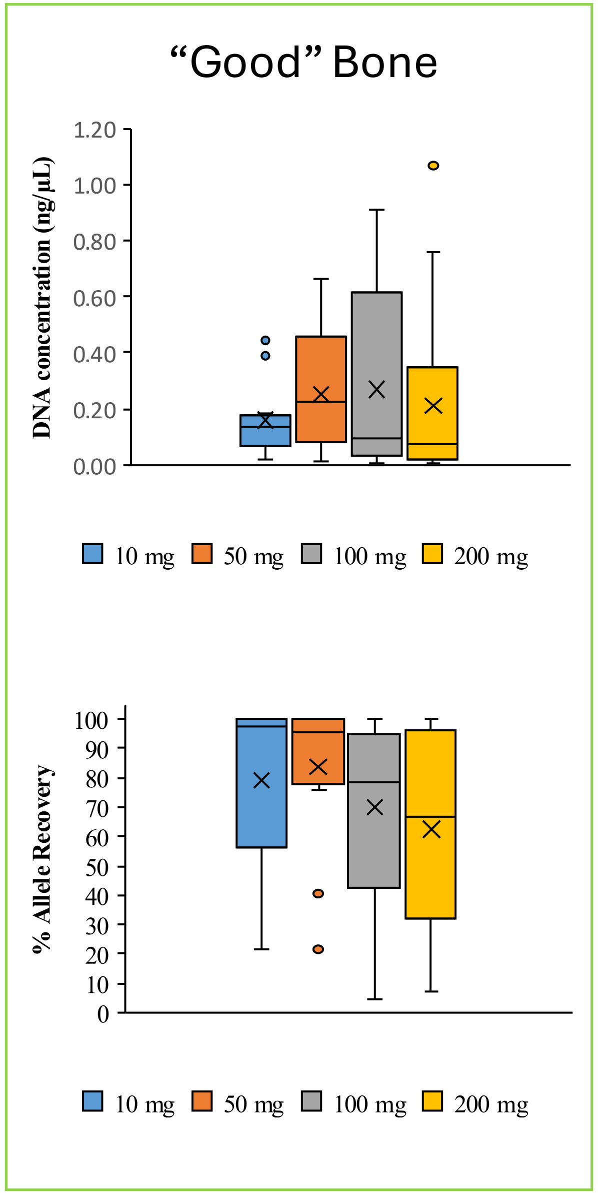
“Compromised” Bone

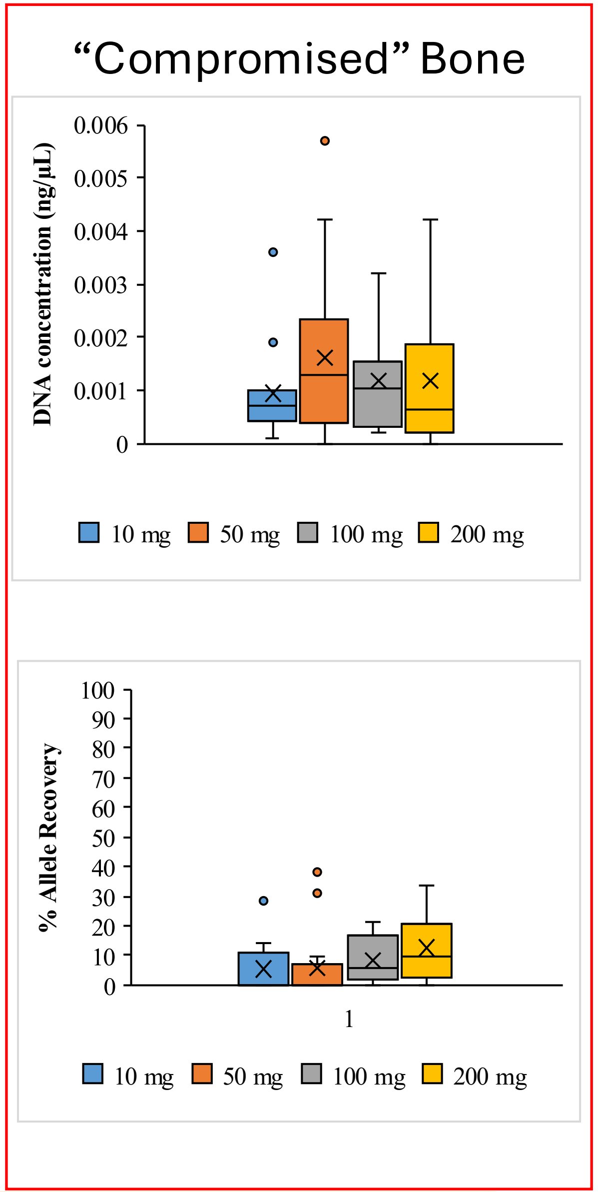


Comparison of Bone Inputs

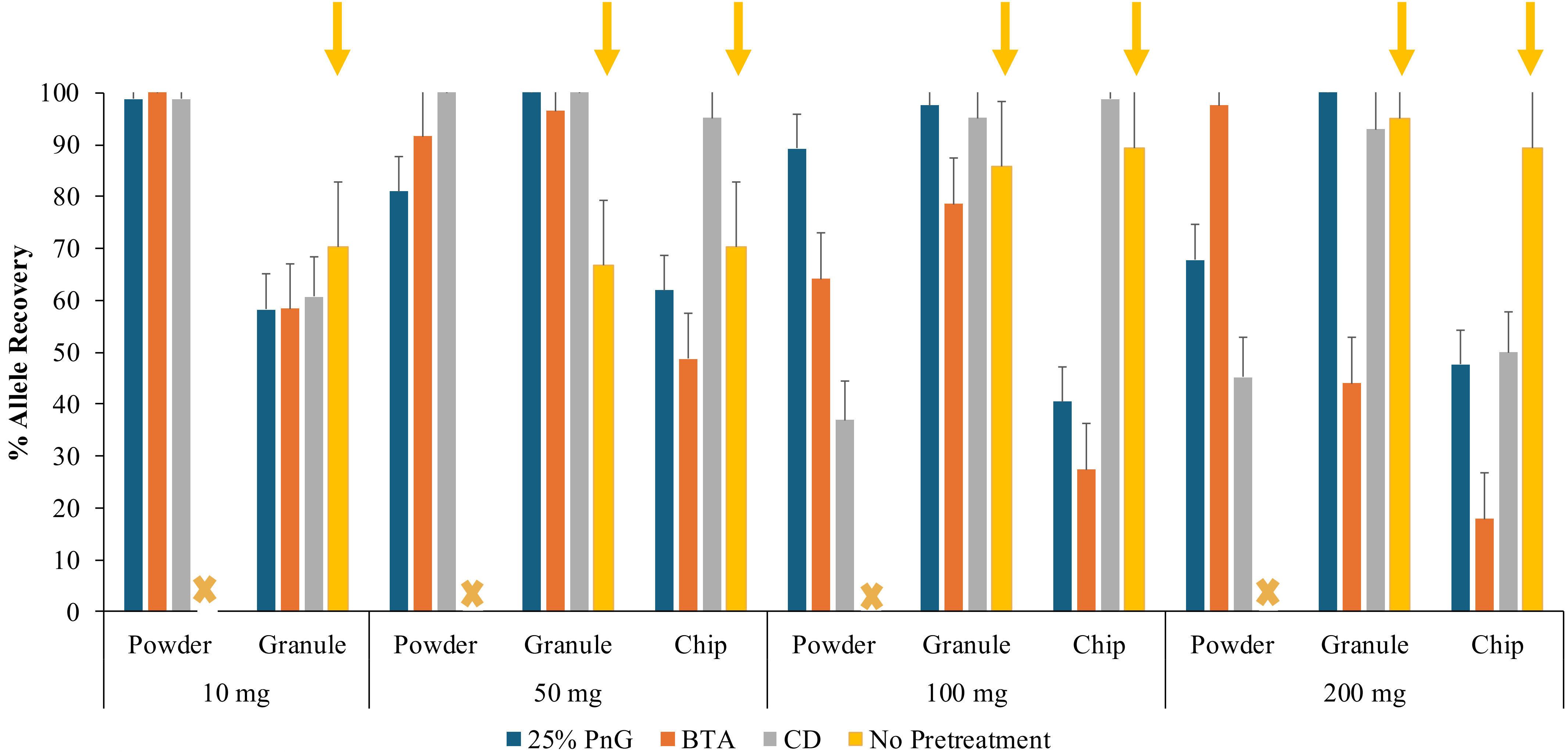
- Regardless of pretreatment, the amount of starting bone mass did not make any statistically significant difference to DNA yield or STR recovery
- Only 5 µL was inputted into our mock rapid method

Bone Amount	Powder (µL)	Spice Grinder (µL)	Chips (µL)
10 mg	100	100	N/A
50 mg	200	200	200
100 mg	400	300	300
200 mg	600	400	400


“Good” Bone


“Compromised” Bone

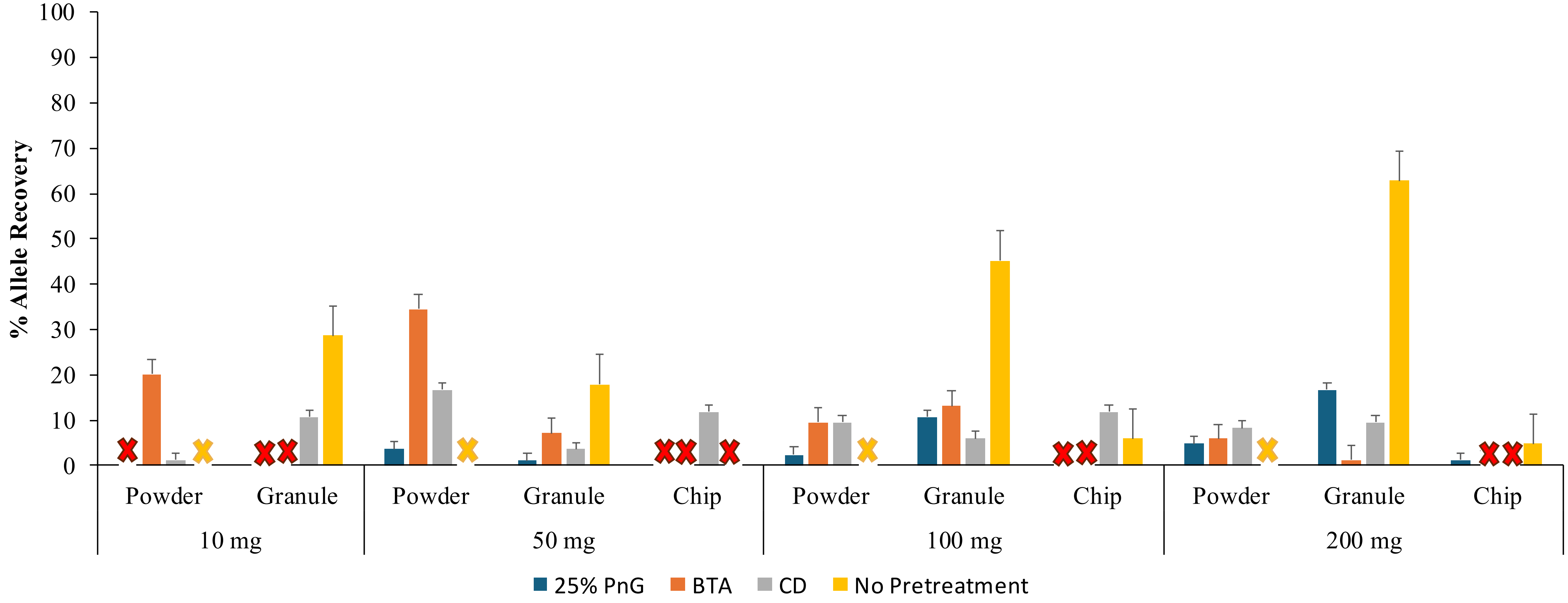
Allele Recovery Summary for “Good” Bone



No powder tested for no treatment
Performed in duplicate; error bars represent standard error

No pretreatment samples yielded only near-complete profiles

Allele Recovery Summary for “Compromised” Bone



No powder tested for no treatment
Performed in duplicate, error bars represent standard error

Pretreatment did not improve STR recovery

Testing the “Mock Rapid” Method

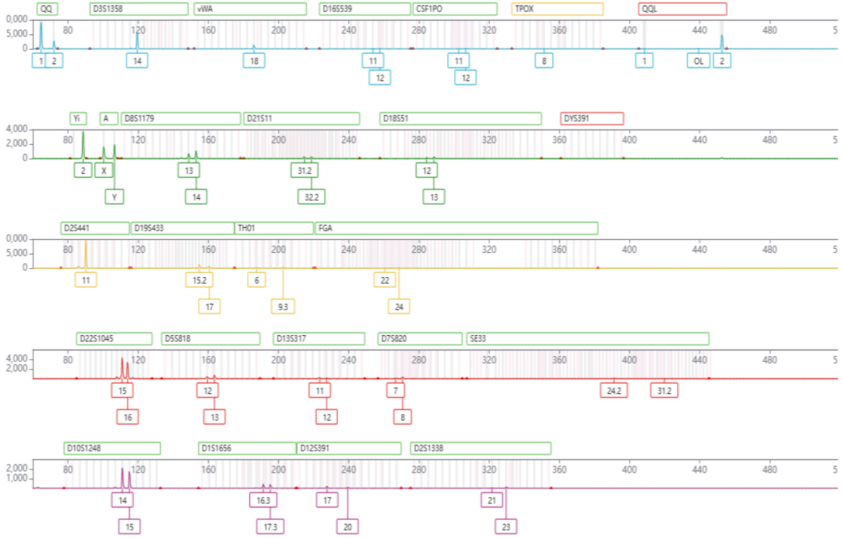
Mock Rapid

- Allele Recovery: 97.6%
- DNA input from Trio: 1.93 ng
- DI value: 126

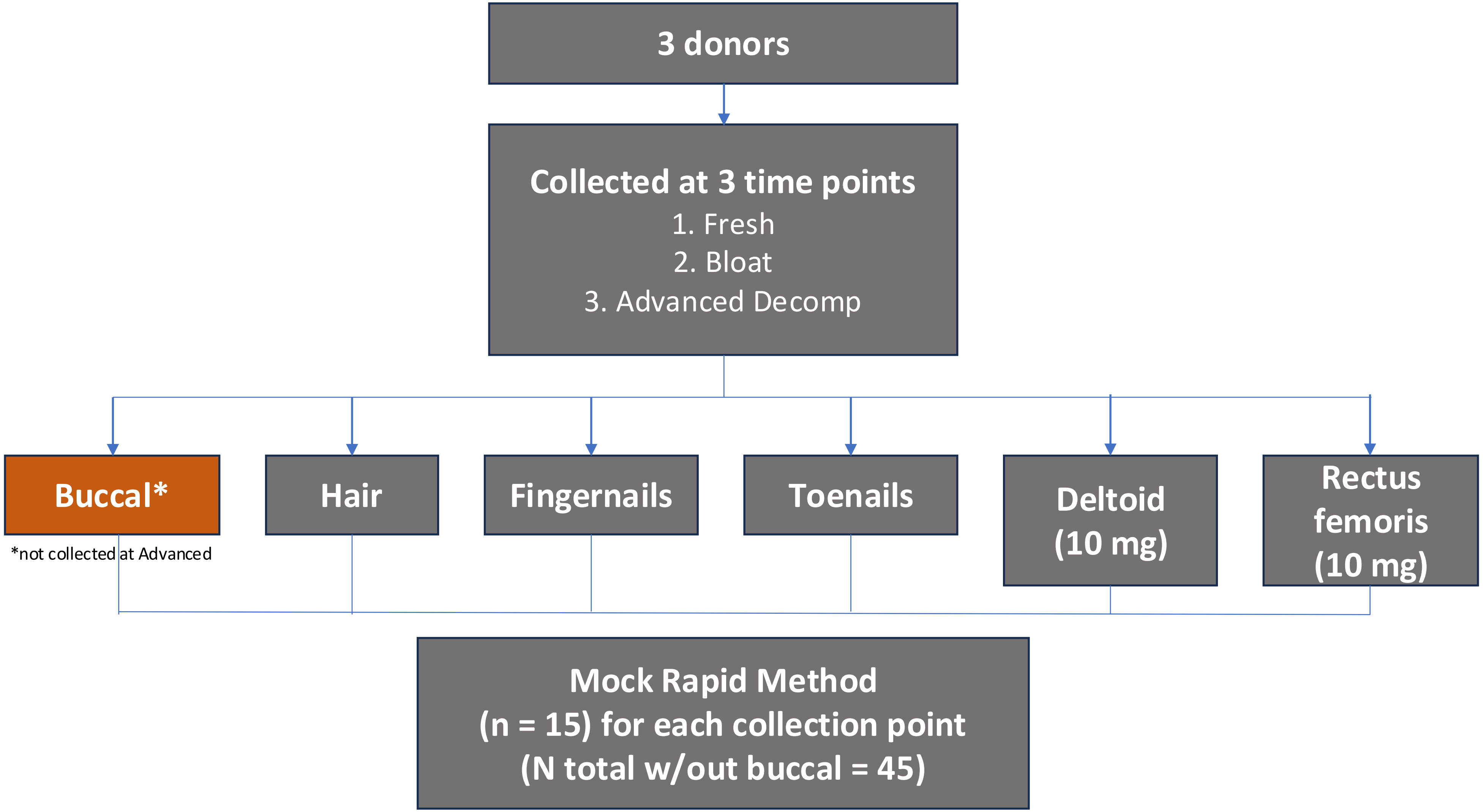
RHID

- Allele Recovery: 95.2%
- DNA input: 4.99 ng
- Degradation flagged

🟩 SQ: 1 🔴 CGQ 🔴 STO ⚠ DEG | QTS: 4990 pg | QTL: 68 pg



Workflow Cadaveric Samples



3 donors

Collected at 3 time points

1. Fresh
2. Bloat
3. Advanced Decomp

Buccal*

Hair

Fingernails

Toenails

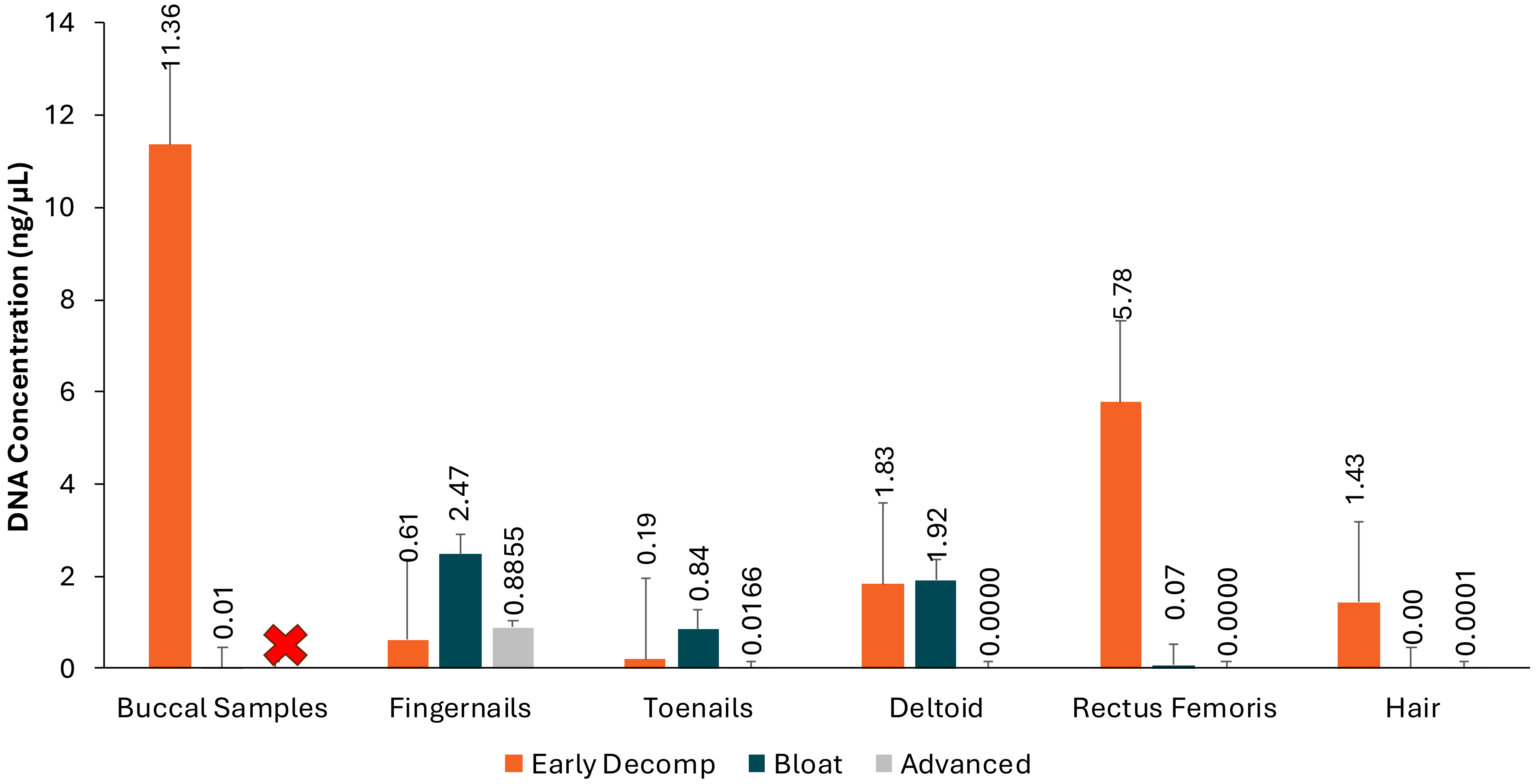
Deltoid (10 mg)

Rectus femoris (10 mg)

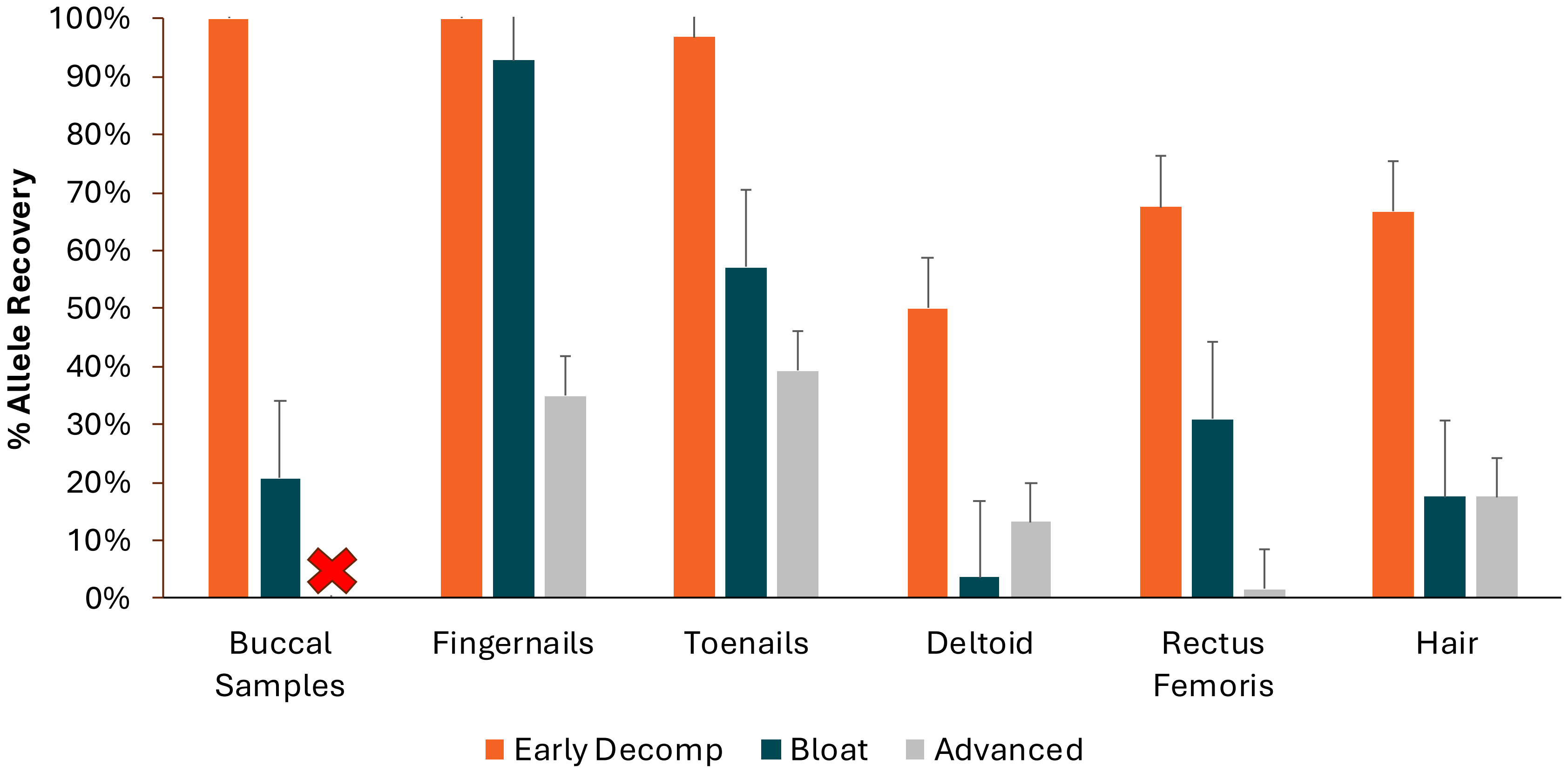
*not collected at Advanced

Mock Rapid Method
(n = 15) for each collection point
(N total w/out buccal = 45)

Quantification Data for Cadaveric Samples
Mock Rapid Method



CE Results for Cadaveric Samples Mock Rapid Method



Nails best for Bloat

Conclusions

Bone Pretreatment

- Spice grinder is the way to go
  - Saves time and resources compared to powdering
- May increase recovery with 25% Prep-n-Go buffer, but pretreatment may not always be necessary

Cadaveric Samples

- Buccal samples and nails are viable options for up to the Bloat phase
- Future work should test more samples
- Once in the Advanced phase, traditional methods should be performed
- Rapid is a valuable tool, but traditional methods might be the best

Mock rapid method serves as an option for triaging samples to save time and cost

References

1. Law Enforcement Resources. FBI. https://le.fbi.gov/science-and-lab/biometrics-and-fingerprints/codis/rapid-dna#:~:text=Rapid%20DNA%2C%20or%20Rapid%20DNA,any%20human%20intervention%20and%20review
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3. Ramos, Erica, Moreno, Liliana, and Hares Douglas. Results of the 2023 Rapid DNA Multi-laboratory Study – RapidINTEL Plus Sample Cartridge. https://doi.org/10.1016/j.fsir.2024.100396

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Questions

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