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Pressure Cycling Technology Research at UNTCHI

Elizabeth Feller

Purpose

- Compare current SOP methods for gDNA and mtDNA extraction to other, new methods
  - Will the new methods work at a comparable level to the current methods? Will they work better?
    - DNA Yield
    - DNA Quality
  - Other considerations:
    - Reagents (types, amounts, overall cost and safety)
    - Analyst time and labor, overall sample processing time

Barocycling

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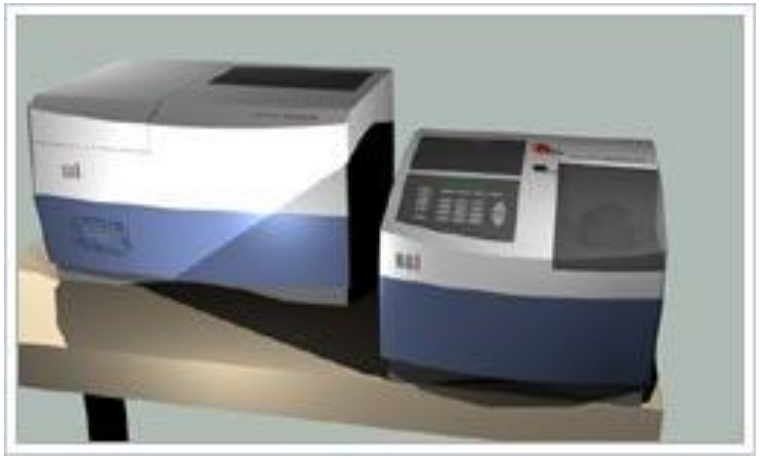
Pressure Cycling Technology (PCT) Extraction Method

30 cycles of:

- 20 sec at 35kpsi
- 10 sec at ambient



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HAIR STUDIES

Phase I Methods

Method 1

Organic Extraction
MicroCon Concentration

Method 2

SEB/ProK Incubation
DNA IQ Extraction and Purification

Method 3

DNA IQ Extraction and Purification

Method 4

SEB/ProK Solution
PCT Extraction
DNA IQ Purification

Method 5

DTT/ProK Solution
PCT Extraction
DNA IQ Purification

Method 6

Promega® Lysis Buffer
PCT Extraction
DNA IQ Purification

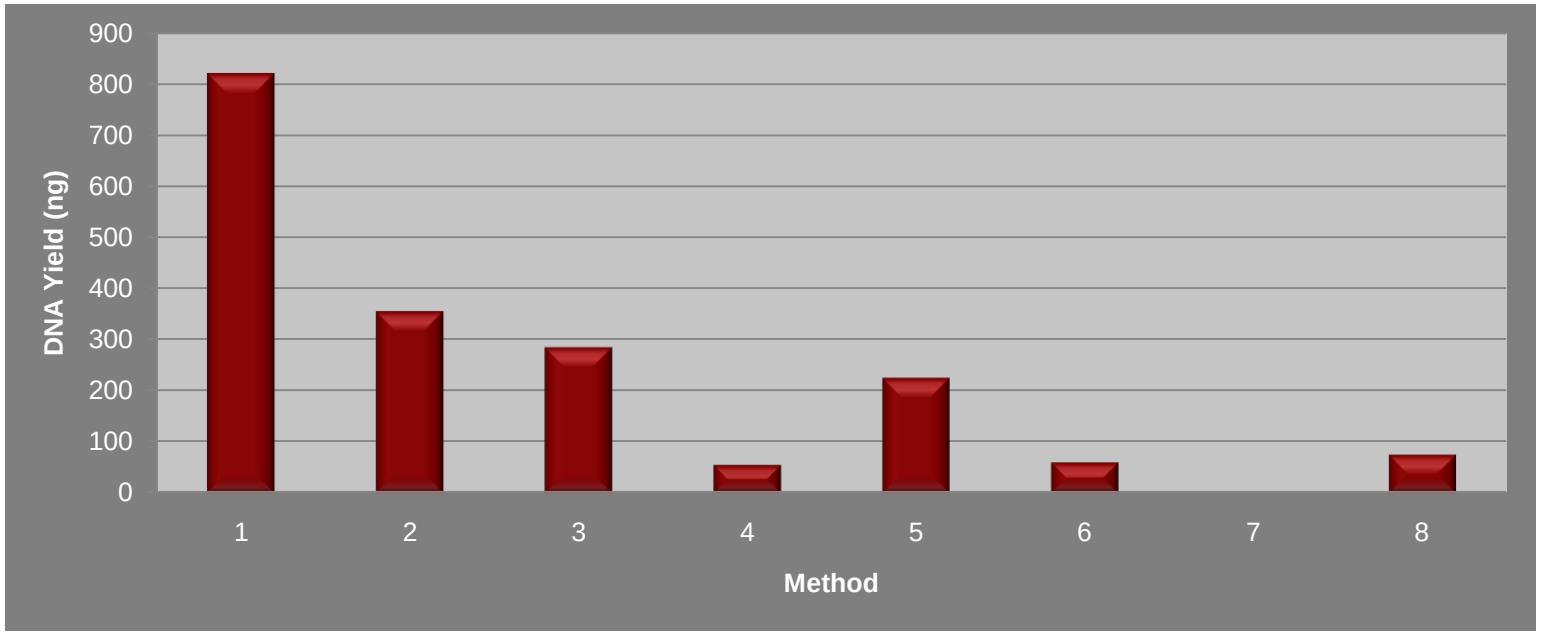
Method 7

Promega® Lysis Buffer/DTT/ProK Solution
PCT Extraction
DNA IQ Purification

Method 8

TE^{-4} Buffer
PCT Extraction
DNA IQ Purification

Average DNA Yields



Legend

Method 1	Organic Extraction with MicroCon Concentration
Method 2	SEB/ProK Incubation with DNA IQ Purification
Method 3	DNA IQ Extraction
Method 4	PCT Extraction with SEB Buffer and DNA IQ Purification
Method 5	PCT Extraction with DTT/ProK Solution and DNA IQ Purification
Method 6	PCT Extraction with Promega Lysis Buffer* and DNA IQ Purification
Method 7	PCT Extraction with Promega Lysis Buffer*/DTT/ProK Solution and DNA IQ Purification
Method 8	PCT Extraction with TE^{-4} Buffer and DNA IQ Purification
SEB	Working Stain Extraction Buffer (with DTT added)
PCT	Pressure Cycling Technology Protocol (30 cycles of: 20s at 35kpsi, 10s at ambient)
*Note:	Promega Lysis Buffer is 1% 1M DTT for Working Solution

Phase I Conclusions

- Of the PCT methods, Method 5 performed best
  - DTT/ProK solution only, no incubation
  - Method 7 produced no results
    - Troubleshooting is ongoing to determine why the extraction or amplification failed
    - Update: Promega® Lysis Buffer contains a Proteinase K inhibitor

Next Steps

- Sequence all samples to assess mtDNA quality
- Determine which, if any, PCT method is most comparable to current methods
  - Should an incubation step be added?
  - Should PCT be performed before or after?
- Update: most samples had interpretable mtDNA sequence for HVI in both directions

Contamination

- Some experiments resulted in contamination in reagent blanks and mixtures in samples
- Traced back to analyst and to PCT tubes
  - Not cleaned before packaging
  - Samples processed in general work area

- Resolution
  - Autoclaving followed by bleaching, washing, and UV irradiation, and working in a “low-copy” area resulted in removal of all contaminants

Phase II Methods

- Methods redesigned to better limit variables
  - 10 methods tested
    - 4 comparing organic extraction
    - 6 comparing DNA IQ extraction
  - Hairs used from 3 unrelated individuals
    - Labeled A, B, and C
  - Fourth individual also tested (D)
    - Methods 5-8, 10

Phase II Methods

Method 1

Organic Extraction
MicroCon Concentration

Method 2

Organic Extraction
PCT after incubation

Method 3

Organic Extraction
PCT before incubation

Method 4

Organic Extraction
PCT with no incubation

Method 5

DNA IQ Extraction

Method 6

DNA IQ Extraction
PCT after incubation

Method 7

DNA IQ Extraction
PCT before incubation

Method 8

DNA IQ Extraction
PCT with no incubation

Method 9

DNA IQ Extraction (Promega® Lysis Buffer only into PCT)
PCT with no incubation

Method 10

DNA IQ Extraction (TE^{-4} Buffer only into PCT)
PCT with no incubation

mtDNA PCR Product Yields

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mtDNA PCR Product Yield from HVI Amplification

(reported in ng/µL)

Method / Hair	1	2	3	4	5	6	7	8	9	10
A	0.13	1.35	0	0	0	3.25	0	0	0.03	0
B	0.29	0	1.96	0.22	0	0	0.02	0	0	0
C	0.3	0.18	0	0.66	0.06	0	0	0.14	0.02	0.2
D	-	-	-	-	1.74	2.87	1.38	0	-	0

Legend

Method 1	Organic Extraction (with MicroCon Concentration)
Method 2	Organic Extraction, PCT after incubation
Method 3	Organic Extraction, PCT before incubation
Method 4	Organic Extraction, PCT with no incubation
Method 5	DNA IQ Extraction and Purification
Method 6	DNA IQ Extraction, PCT after incubation
Method 7	DNA IQ Extraction, PCT before incubation
Method 8	DNA IQ Extraction, PCT with no incubation
Method 9	PCT Extraction with Promega Lysis Buffer* and DNA IQ Purification
Method 10	PCT Extraction with TE^{-4} Buffer and DNA IQ Purification
PCT	Pressure Cycling Technology Protocol (30 cycles of: 20s at 35kpsi, 10s at ambient)
*Note:	Promega Lysis Buffer is 1% 1M DTT for Working Solution

Phase II Conclusions

- mtDNA PCR product yield inconsistent
  - Between individuals and from one individual
  - Future studies may need to use triplicate hairs from one individual for each method, instead of single hairs from multiple individuals
- Overall low yields
  - Mostly thin hairs collected
- No instances of contamination!
- Possibly investigate PCT reactions using reduced volumes in MicroTubes

Phase III Methods

- Testing more specific methods based on results of phases I and II and incorporating MicroTubes
  - 10 methods tested
    - 5 comparing organic extraction
    - 5 comparing DNA IQ extraction
  - Hairs used from 3 unrelated individuals
    - Labeled A, B, and C

Phase III Methods

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Method 1

Organic Extraction
MicroCon Concentration

Method 2

Organic Extraction
PCT after incubation
MicroCon Concentration

Methods 3A, 3B, and 3C

Organic Extraction
miPCT after incubation
(150µL, 100µL and 50µL respectively)
MicroCon Concentration

Method 4

DNA IQ Extraction

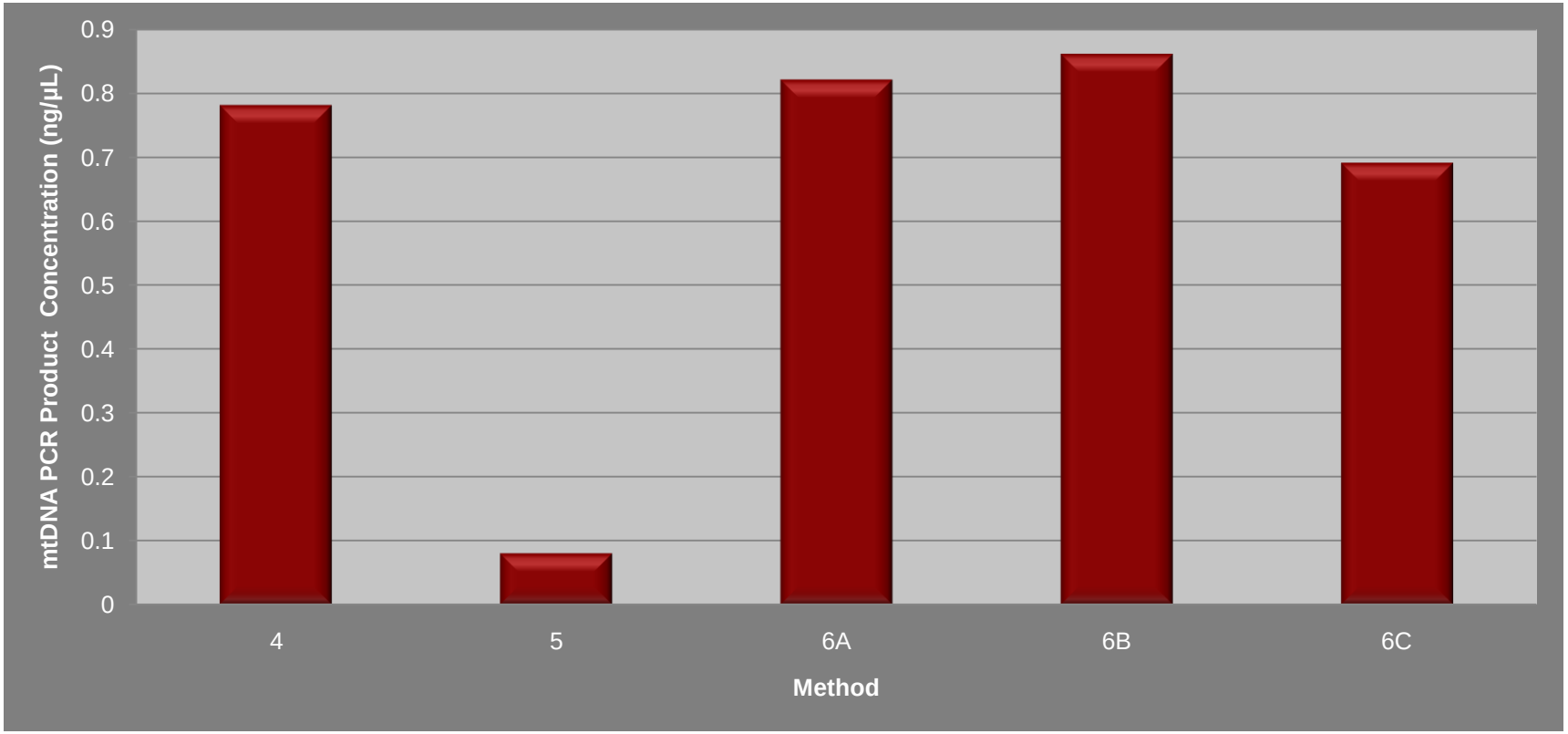
Method 5

DNA IQ Extraction
PCT after incubation

Methods 6A, 6B, and 6C

DNA IQ Extraction
miPCT after incubation
(150µL, 100µL, and 50µL, respectively)

Average mtDNA PCR Product Concentration



Legend

Method 4	DNA IQ Extraction and Purification
Method 5	DNA IQ Extraction, PCT after incubation
Method 6A	DNA IQ Extraction, miPCT after incubation (150µL)
Method 6B	DNA IQ Extraction, miPCT after incubation (100µL)
Method 6C	DNA IQ Extraction, miPCT after incubation (50µL)
PCT	Pressure Cycling Technology Protocol (30 cycles of: 20s at 35kpsi, 10s at ambient)

Phase III Conclusions

- Most increase in yield observed with methods that use PCT at 100µL reaction volumes
  - Best yield: Method 6B (DNA IQ with miPCT at 100µL)
- MicroTubes can successfully be used to extract mtDNA from hairs
- Wider variety of hairs should be tested
  - Will texture and pigmentation differences result in differences in extraction success with PCT?
  - Will observed yields be consistent across a broader spectrum of hair types?

Phase IV Methods

- Testing hairs from 20 individuals of varying nationality and hair types
- Narrowed methods to the PCT methods that have been most consistent to compare to current procedures

Phase IV Methods

Method 1

Organic Extraction
MicroCon Concentration

Method 2

Organic Extraction
PCT after incubation
MicroCon Concentration

Method 3

Organic Extraction
miPCT after incubation (100µL)
MicroCon Concentration

Method 4

DNA IQ Extraction

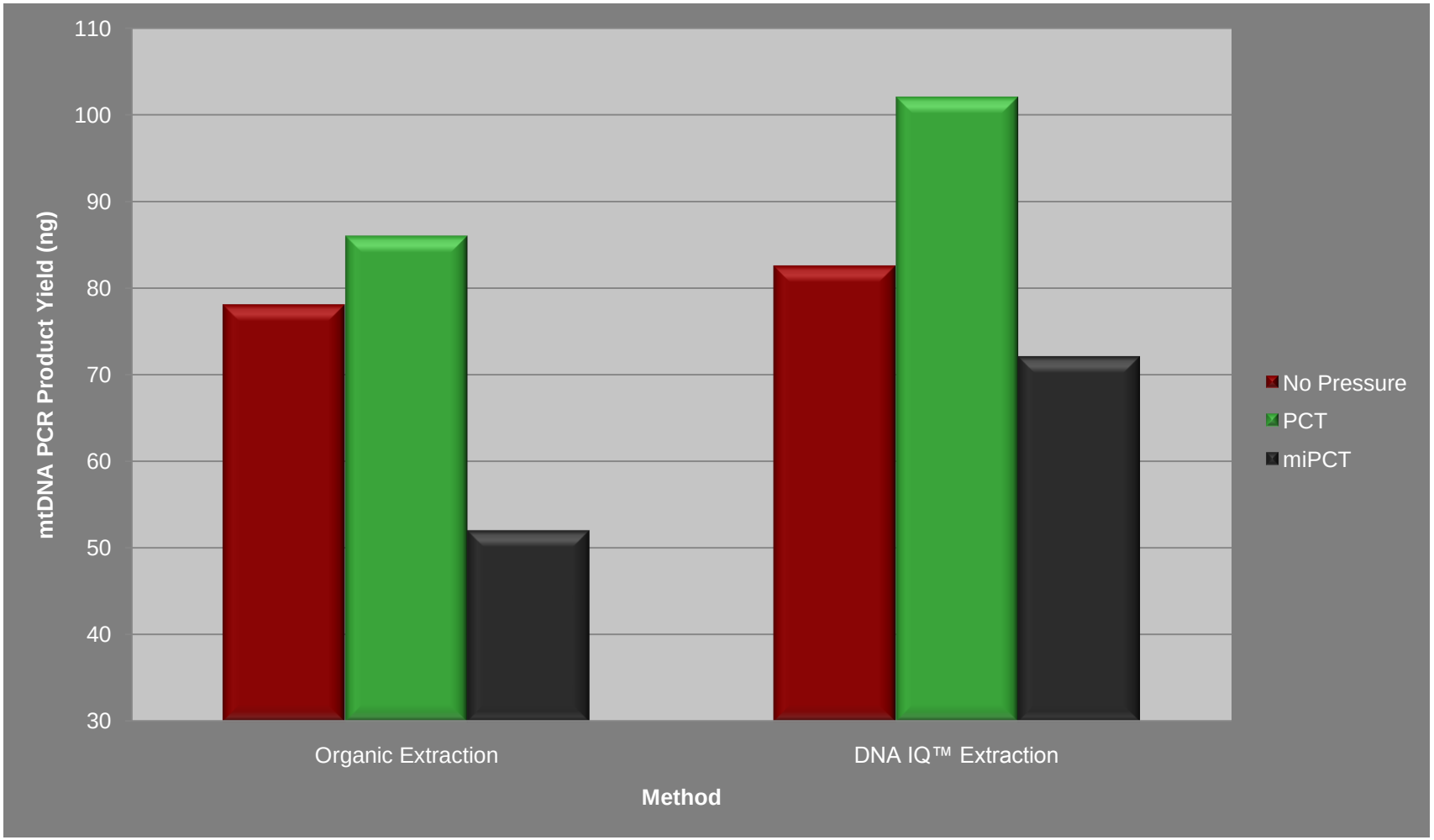
Method 5

DNA IQ Extraction
PCT after incubation

Method 6

DNA IQ Extraction
miPCT after incubation (100µL)

mtDNA PCR Product Yields



Agilent Results

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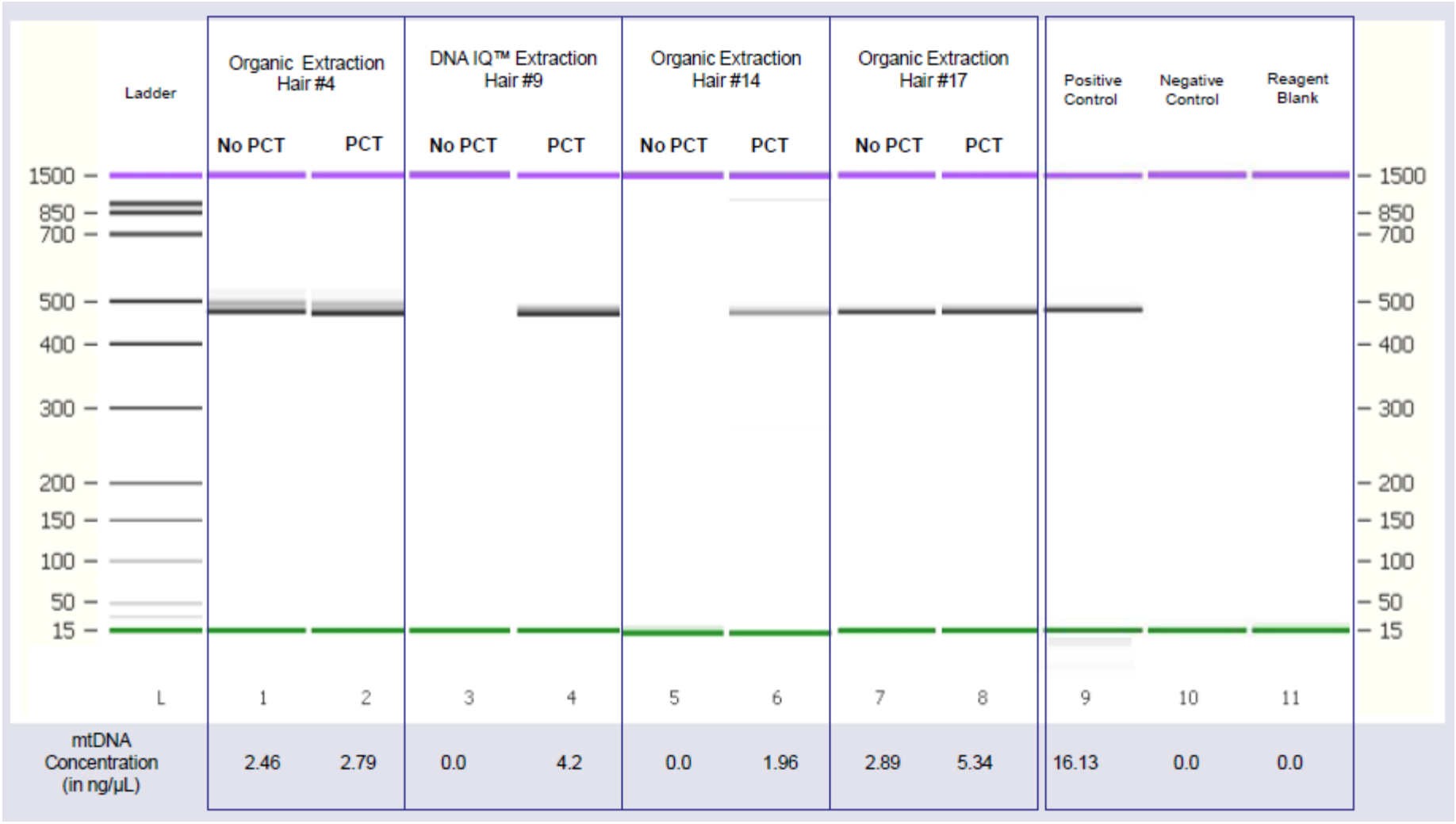

mtDNA Concentration (in ng/µL)	2.46	2.79	0.0	4.2	0.0	1.96	2.89	5.34	16.13	0.0	0.0

Hair #17

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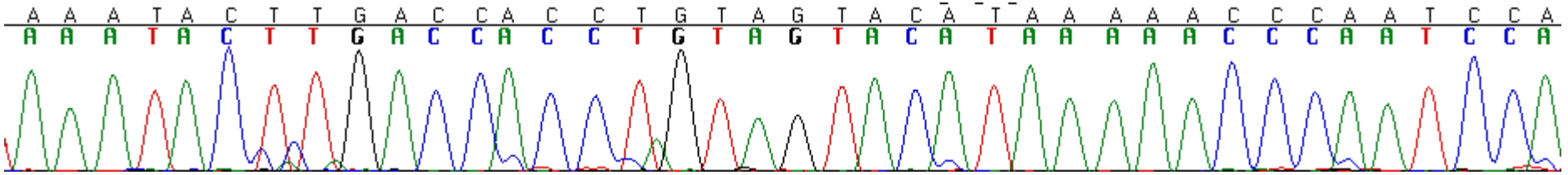
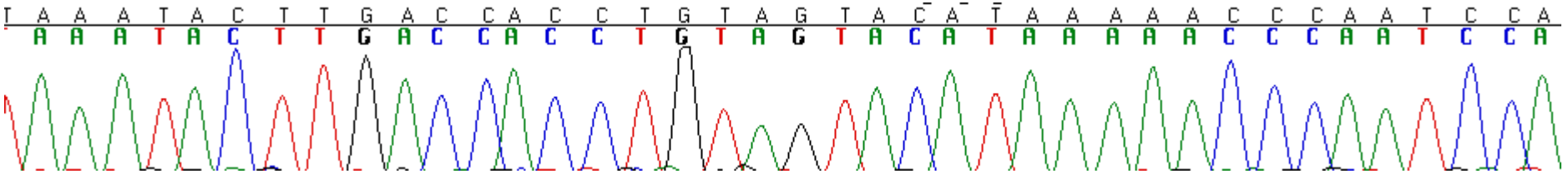
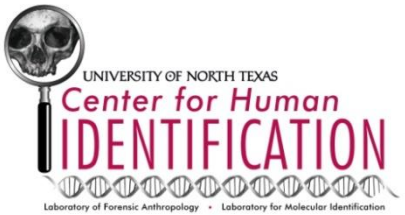
Organic Extraction Without PCT



Organic Extraction With PCT



Conclusions



- PCT can improve mtDNA extraction from hair shafts
- For hair shafts, PCT does not retain sample morphology
- More refinement of the PCT methods may allow for reduced-volume reactions and shorter processing time

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BONE STUDIES

Study Design

- 12 bone samples (0.2g each) incubated with Promega Bone Incubation Buffer and ProK [1mg/mL] for 1hr at 56˚C
  - Bones chosen based on previous testing:
    - 4 bones – full STR profile
    - 4 bones – partial STR profile
    - 4 bones – no STR profile
- Samples processed with or without PCT
- Post-extraction purification using DNA IQ™ Casework Sample Kit on the Maxwell® 16

Average DNA Yields from Bone

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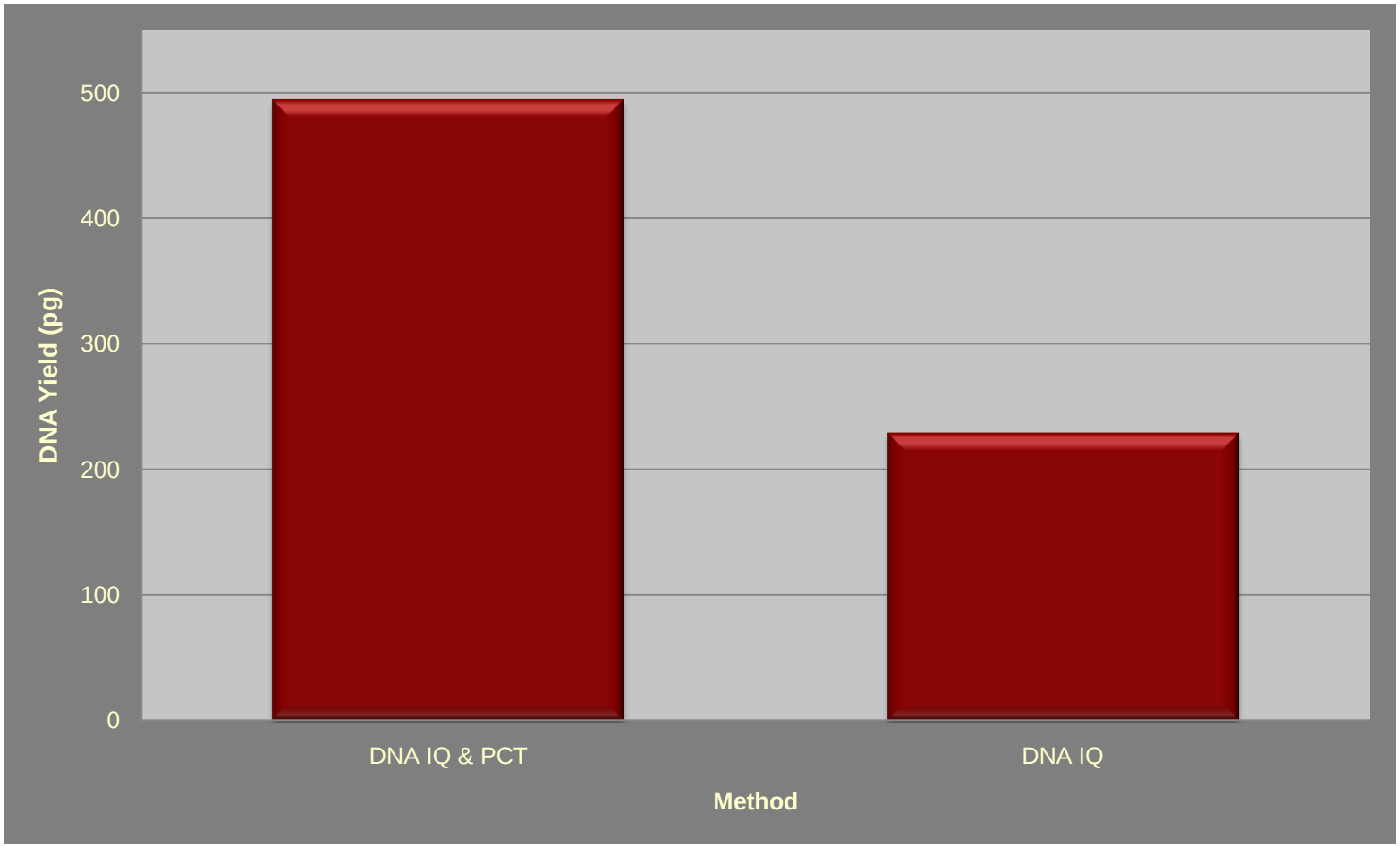

Bone #10: No Pressure

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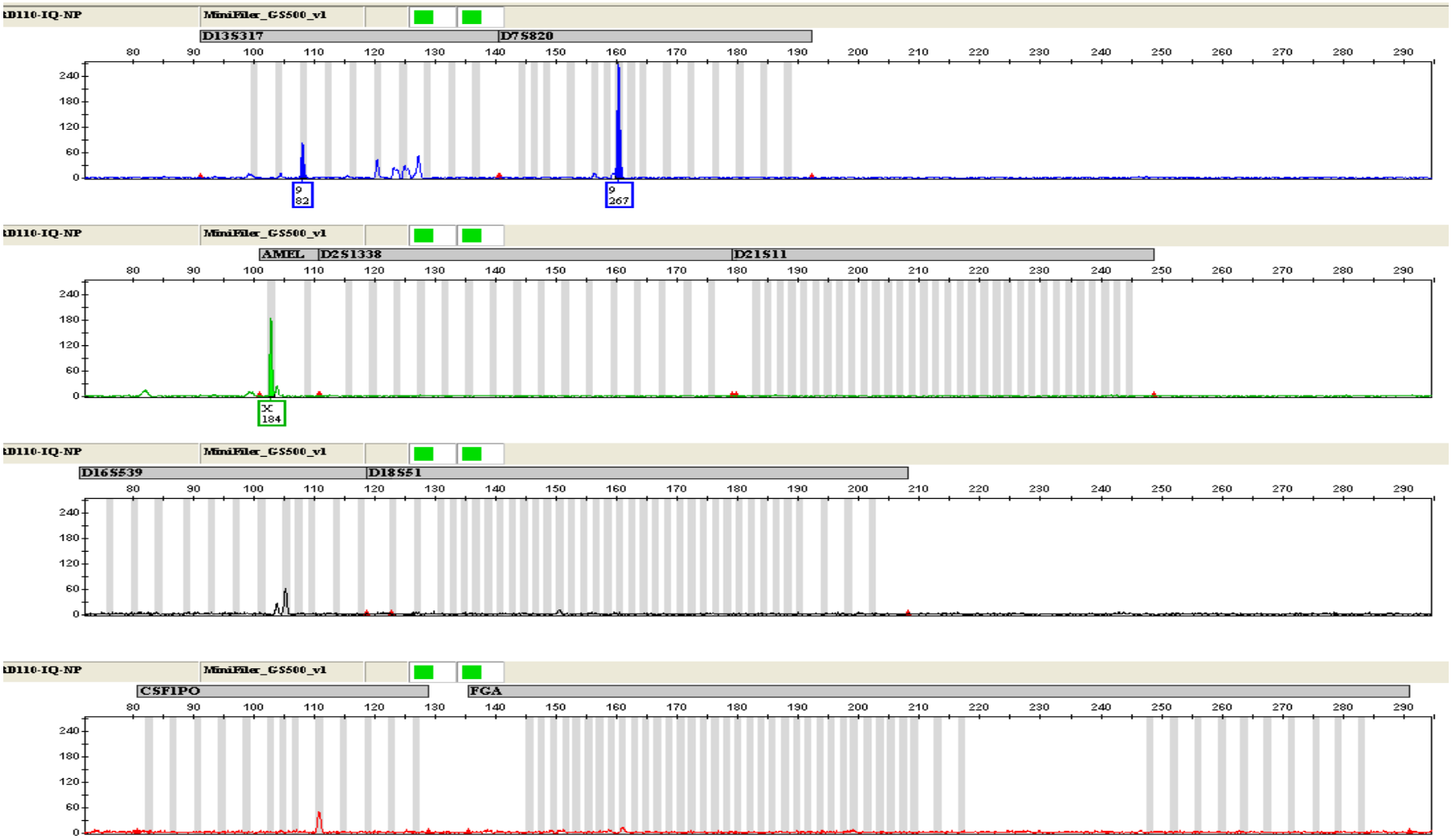

Bone #10: with PCT

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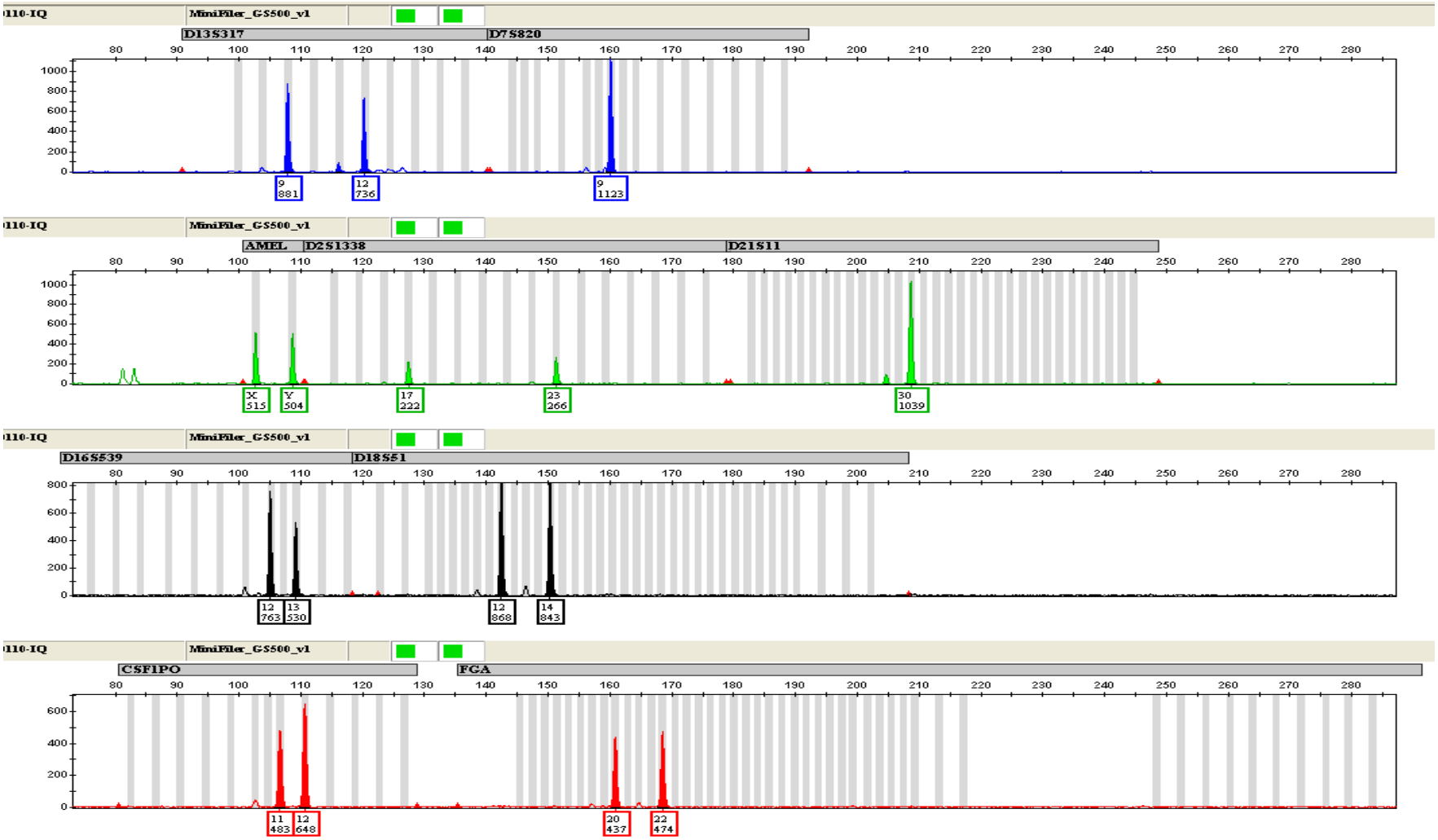

Bone #4: No Pressure

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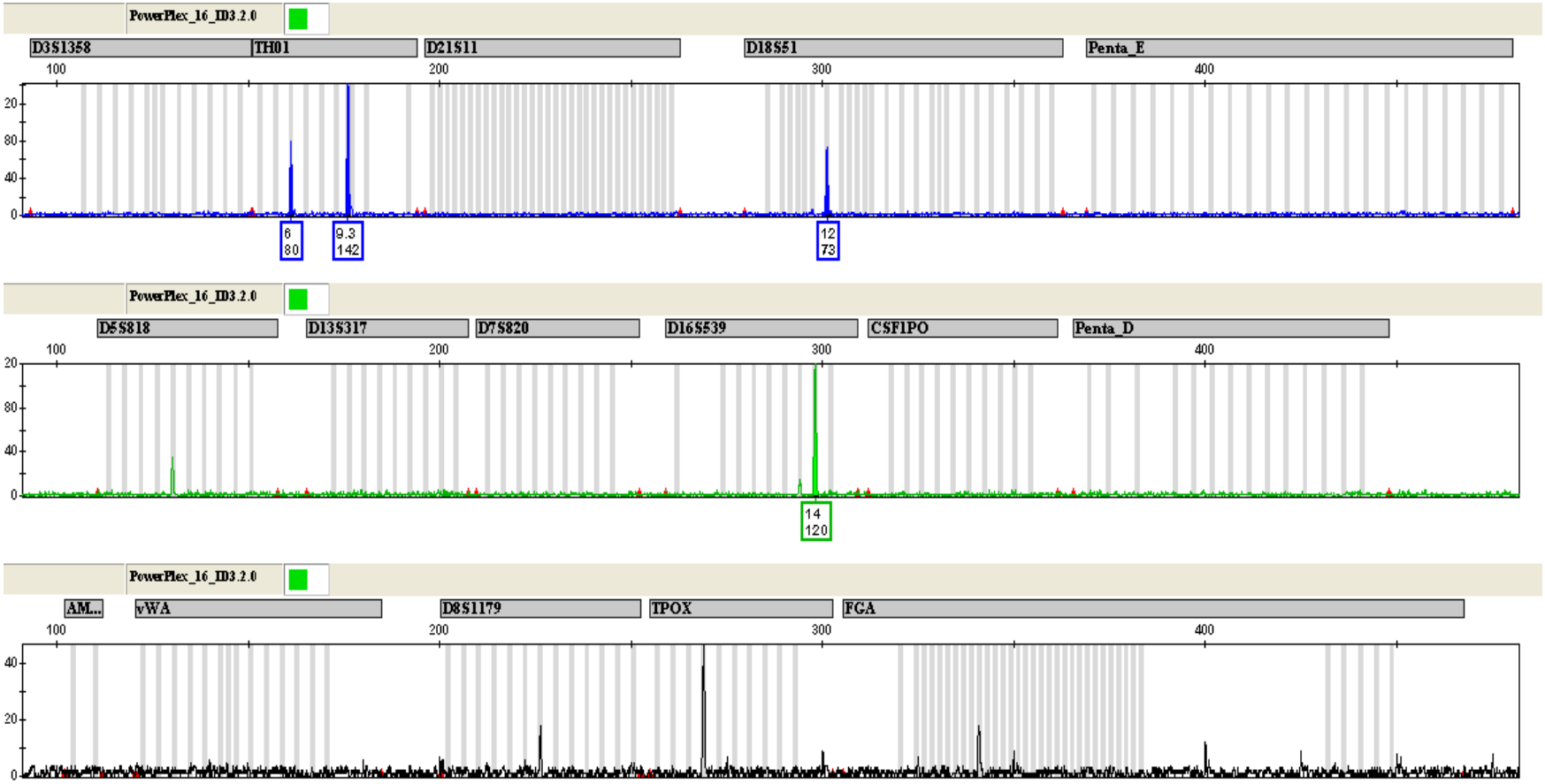

Bone #4: with PCT

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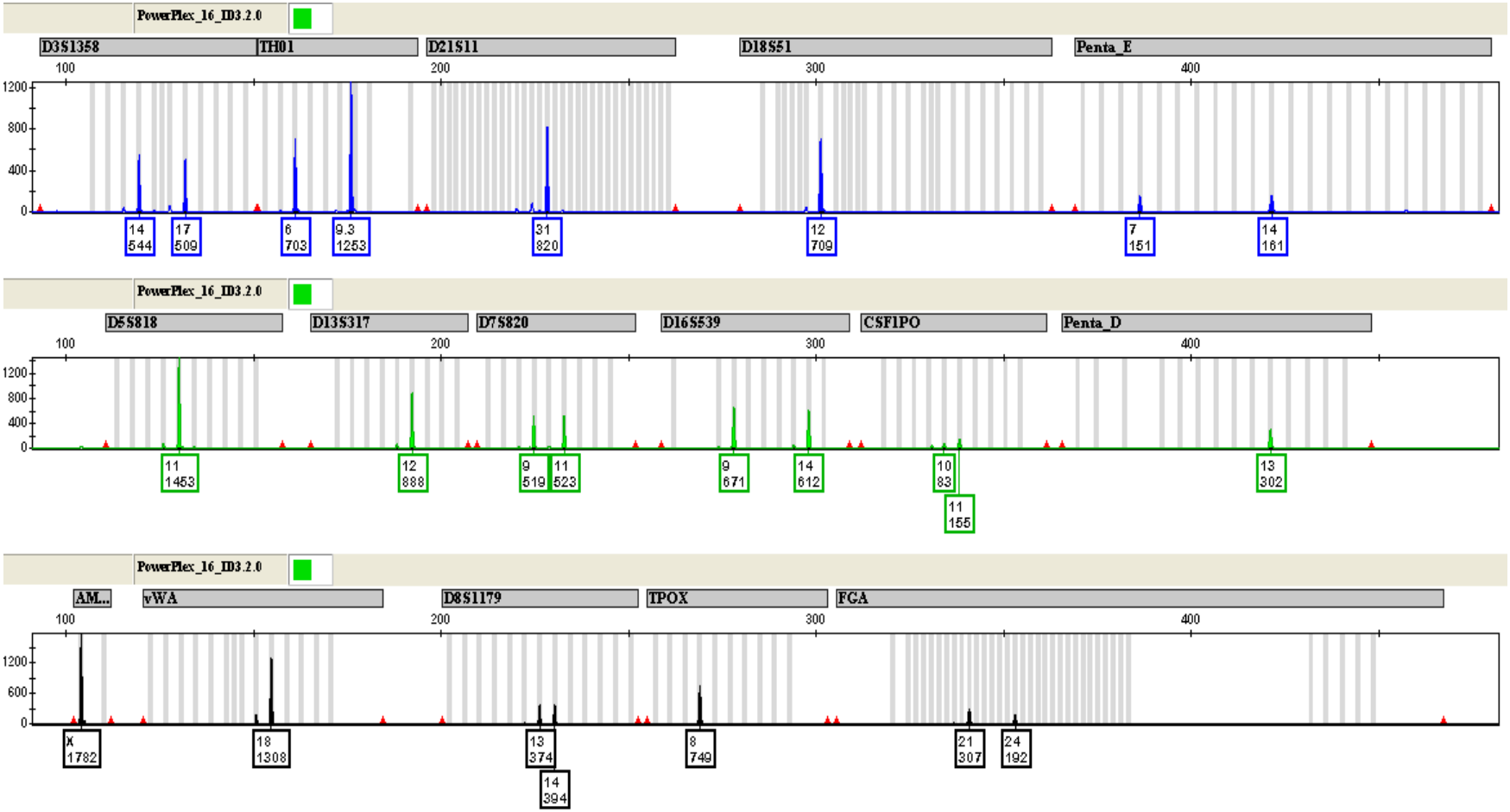

Bone #1

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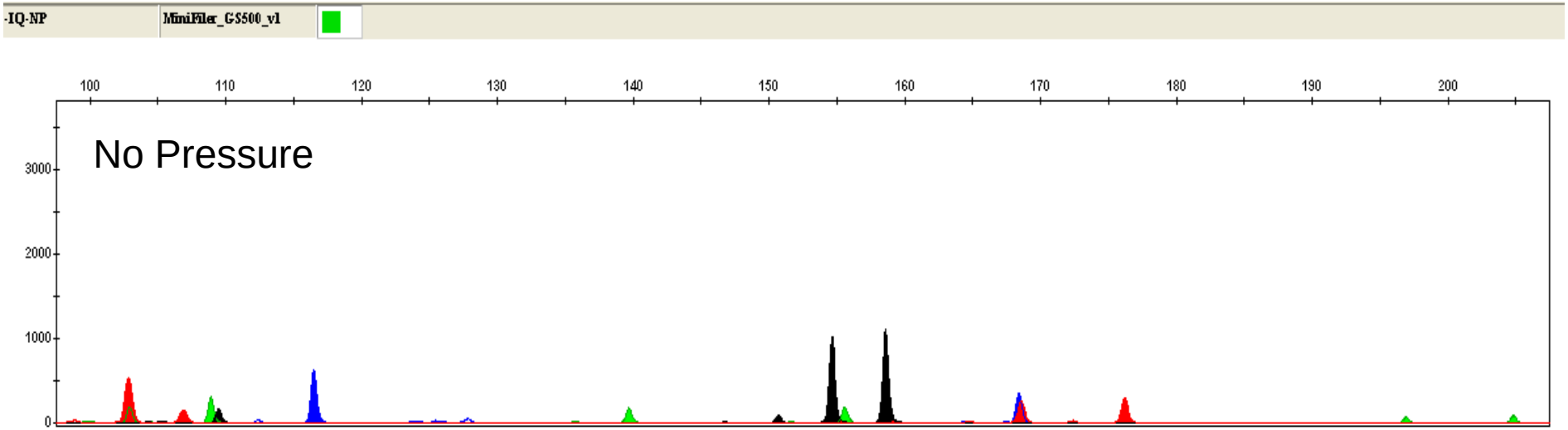

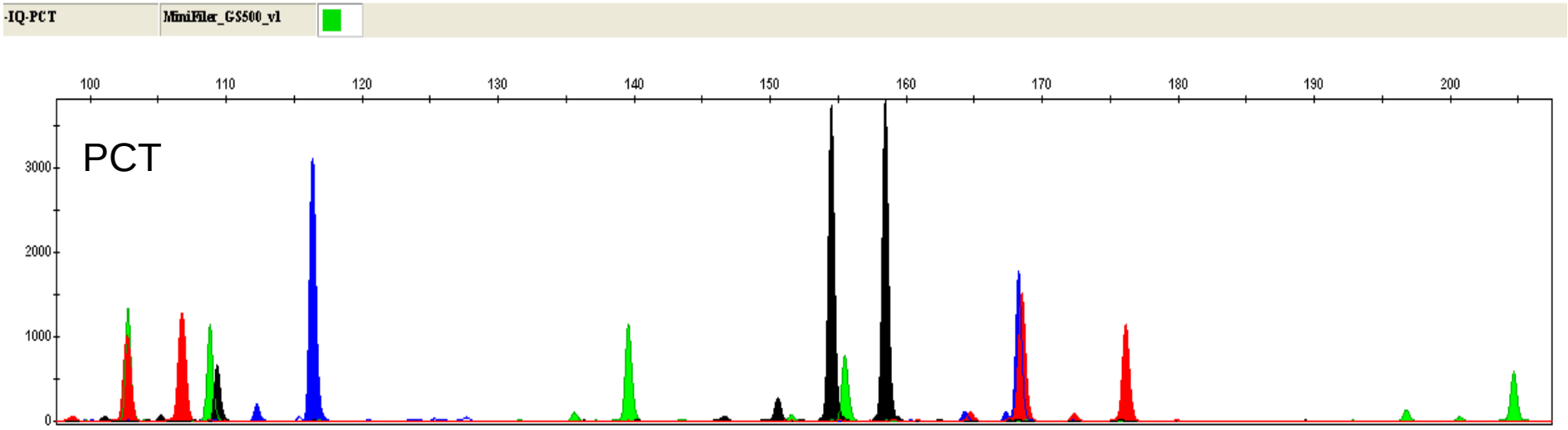

Bone #5

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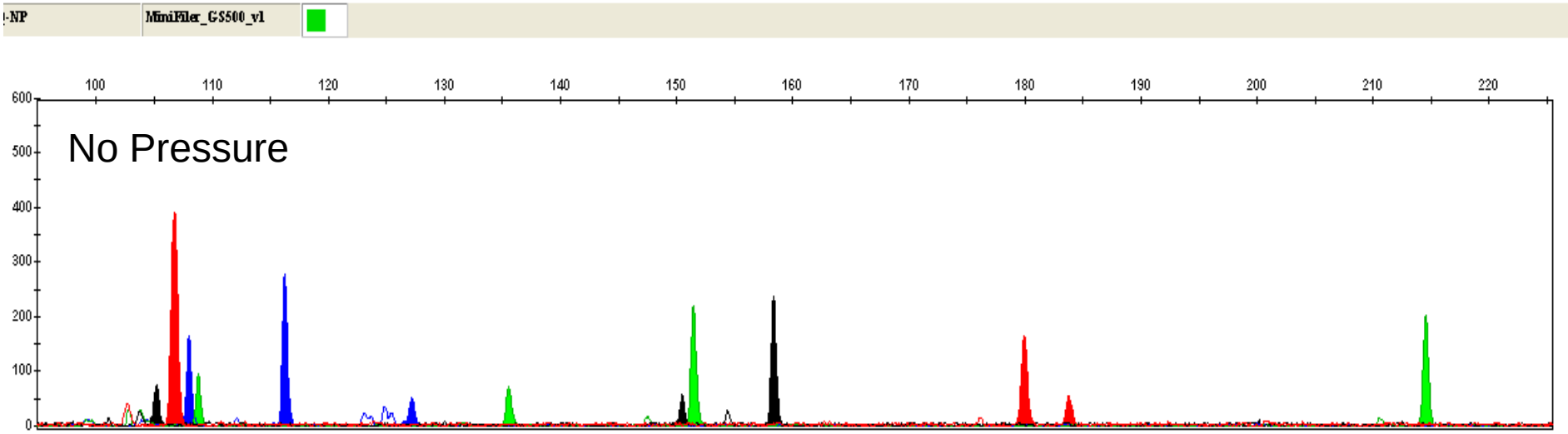

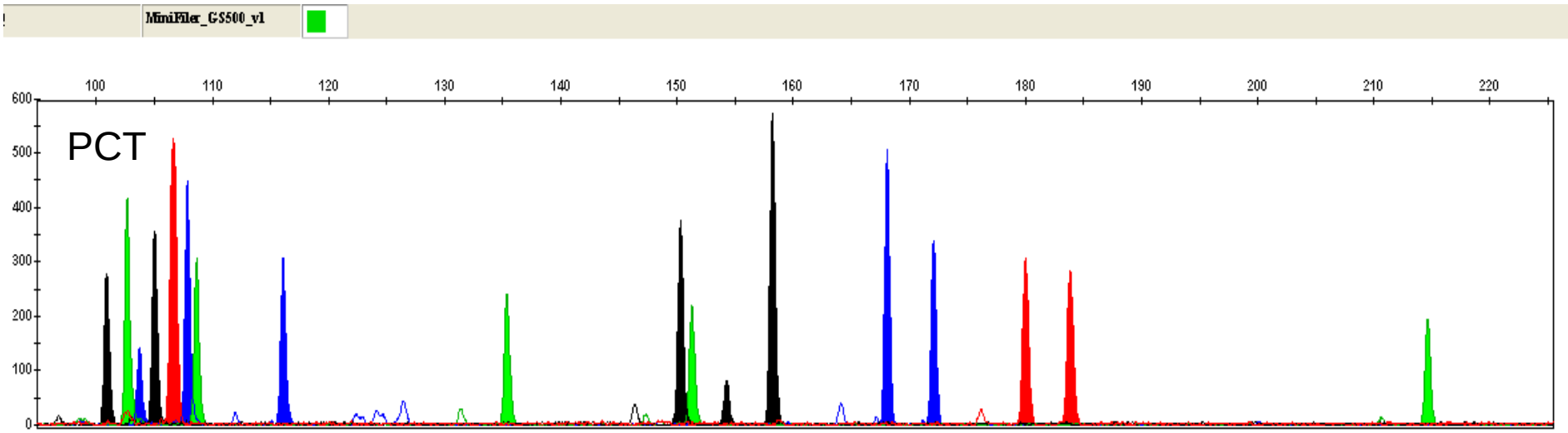

Conclusions

- PCT treatment resulted in slightly improved STR profiles (not significant) than standard method for some bones
- However, PCT should be used as an additional tool for bone analysis only
  - Variations in bones themselves factor into extraction success
- PCT is a viable alternative method for DNA extractions
- PCT can be used in conjunction with commercially available extraction reagents
- PCT may effectively release DNA without destroying or consuming the sample (e.g.: bone shards)

Areas of Interest

- DNA extraction from swabs
  - Low-copy
- Optimization of cycling parameters
- Differential extractions

Questions?

Thank you!

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