

Pressure BioSciences, Inc.

Pressure Cycling Technology (PCT):

**Advancements in
Biological Sample Preparation
Potential
Applications in Forensic Science**



Discovery Starts With Sample Preparation

PBI

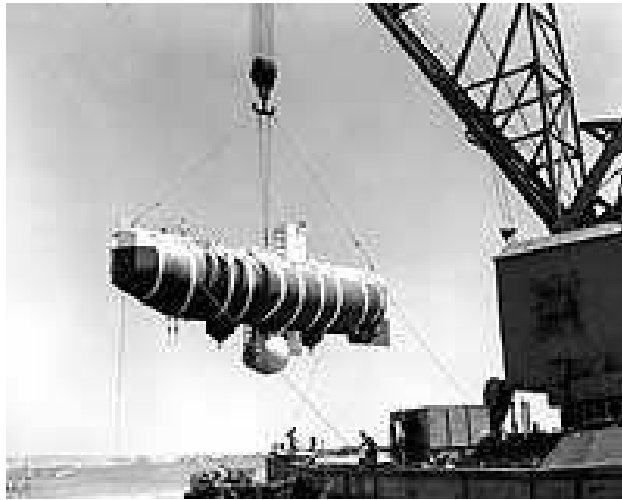
History of High Pressure in Life Sciences

- **1623-1662:** Blaise Pascal – described fundamental concepts of pressure and vacuum
- **1895:** H. Royer – pressure kills bacteria
- **1899:** B.H. Hite *et al.* – pressure preserves milk
- **1914:** P.W. Bridgman - pressure coagulates egg white
- **1989:** High pressure processing of food products
- **2000:** First International Conference on HPBB
- **2008:** Fifth International Conference on HPBB in the USA

Pressure Cycling Technology (PCT)

PCT is a Novel, Enabling Technology that Uses Cycles of Hydrostatic Pressure Between Atmospheric and Ultra-high Levels (up to 35,000 psi and greater) to Allow for the Precise Control of Biomolecular Interactions

Understanding Hydrostatic Pressure



**U.S. Navy Bathyscaphe
Trieste (1958-1963)**



**Marianas Trench:
38,713 ft (11,800m) deep
16,000 PSI (120MPa)**

Significant portion of the Global Biosphere is
subjected to high hydrostatic pressure!

Pressure: a Thermodynamic Process

- Pressure is a measurement of the force exerted per unit area on the boundaries of a substance or system.
- It is caused by the collisions of the molecules of the substance with the boundaries of the system.
- As molecules hit the walls, they exert forces that try to push the walls outward.
- The forces resulting from all these collisions cause the pressure exerted by a system on its surroundings.

Compressibility

- Organic material is more compressible than water
- PCT exploits the differences between compressibility of different components of the sample
- Compression heating is proportional to compressibility
- Hydrostatic pressure does not cause shearing

Synergy of Physics and Chemistry

- PCT selectively disrupts membrane structures based on their size and fluidity
- PCT is compatible with a wide variety of reagents
- Enzymatic lysis is enhanced by Pressure Cycling
- Temperature and pressure are generally synergistic
- PCT can be combined with affinity purification

Current Extraction Methods

- Mortar & Pestle
- Dounce homogenizer (glass on glass)
- Potter-Elvehjem homogenizer (Teflon on glass)
- Enzymatic Digestion
- Polytron shearing homogenizers
- Blenders
- Bead Beating
- Sonication
- Repeated Freeze/Thaw cycles
- French Press (≤ 2000 PSI)



State of the Art?

“A collaborator at a major university in a multi-million dollar Proteomics Facility, equipped with the most advanced instrumentation...

...but they use mortar and pestle.”



Native American Indian
Mortar and Pestle

circa 1000 AD

PCT Sample Preparation System



Hydraulic System
3 Samples Simultaneously
Optional Temperature Control

Barocycler™ NEP3229



PBI

PCT Sample Preparation System



Pneumatic System
Single Sample Capacity
Optional Temperature Control



Barocycler™ NEP2320

User-Adjustable Variables

- Pressure (up to 35 kpsi)
- Number of Cycles
- Cycle Profile
- Chemistry
- Temperature

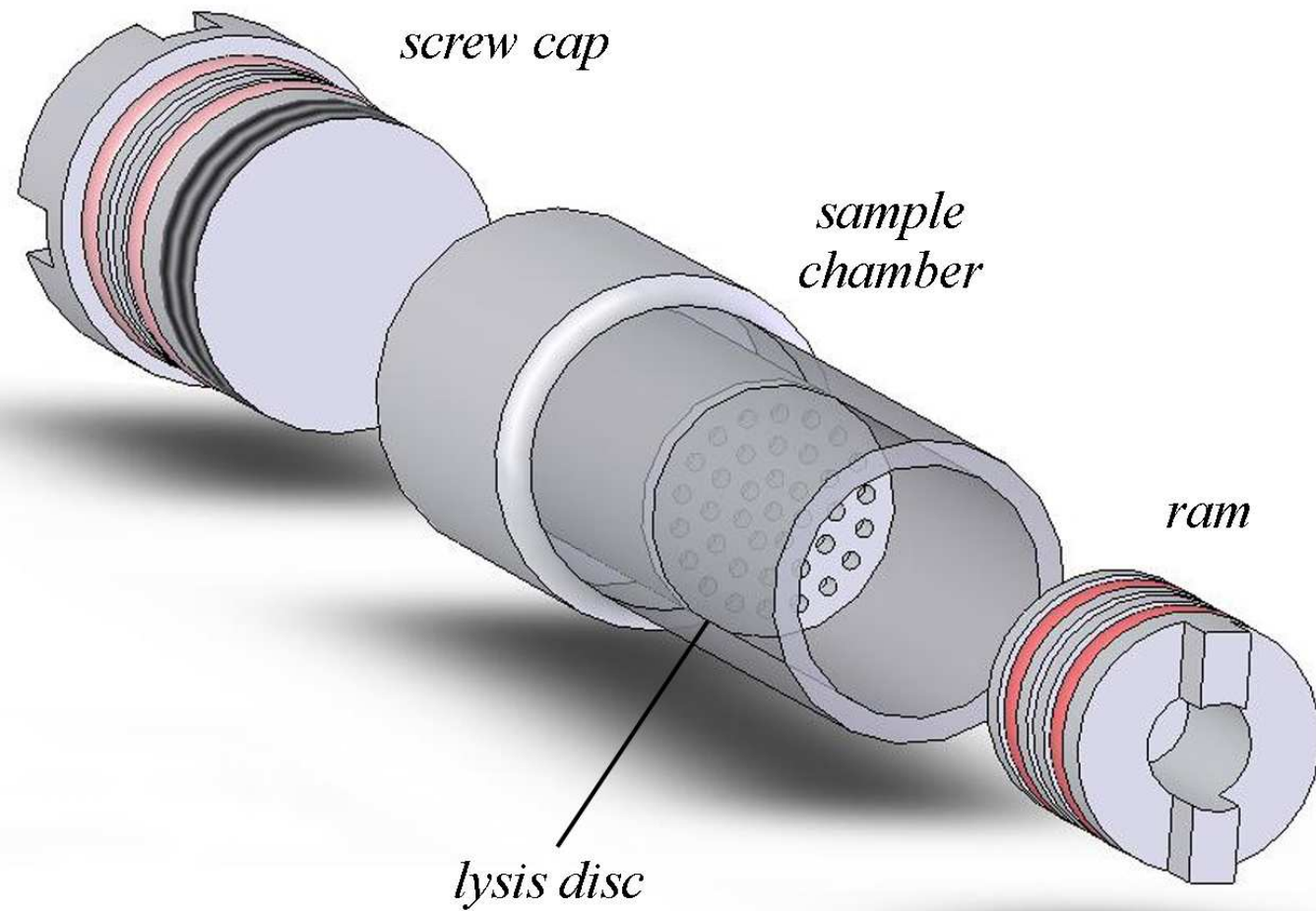
The FT500 PULSE Tube

Specially designed multi-functional tube

- Single-Use
- Versatile, works with:
 - Standard and custom reagents
 - Various sample types
 - Range of sample sizes
- Convenient
- Efficient
- Safe: closed tube, sample fully-contained



The PULSE Tube



Diskless PULSE Tube

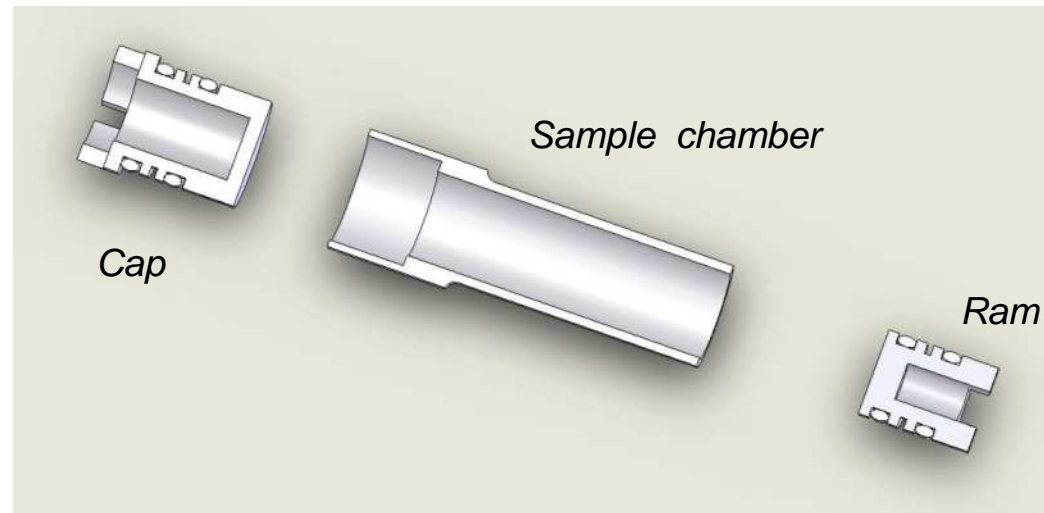


FT500-ND PULSE Tubes

Variable Volume PULSE Tube™ FT 500-ND

100 – 1500 μ L

- Cell Suspensions
- Emulsions
- Soil samples
- In-solution digestion



Mechanical

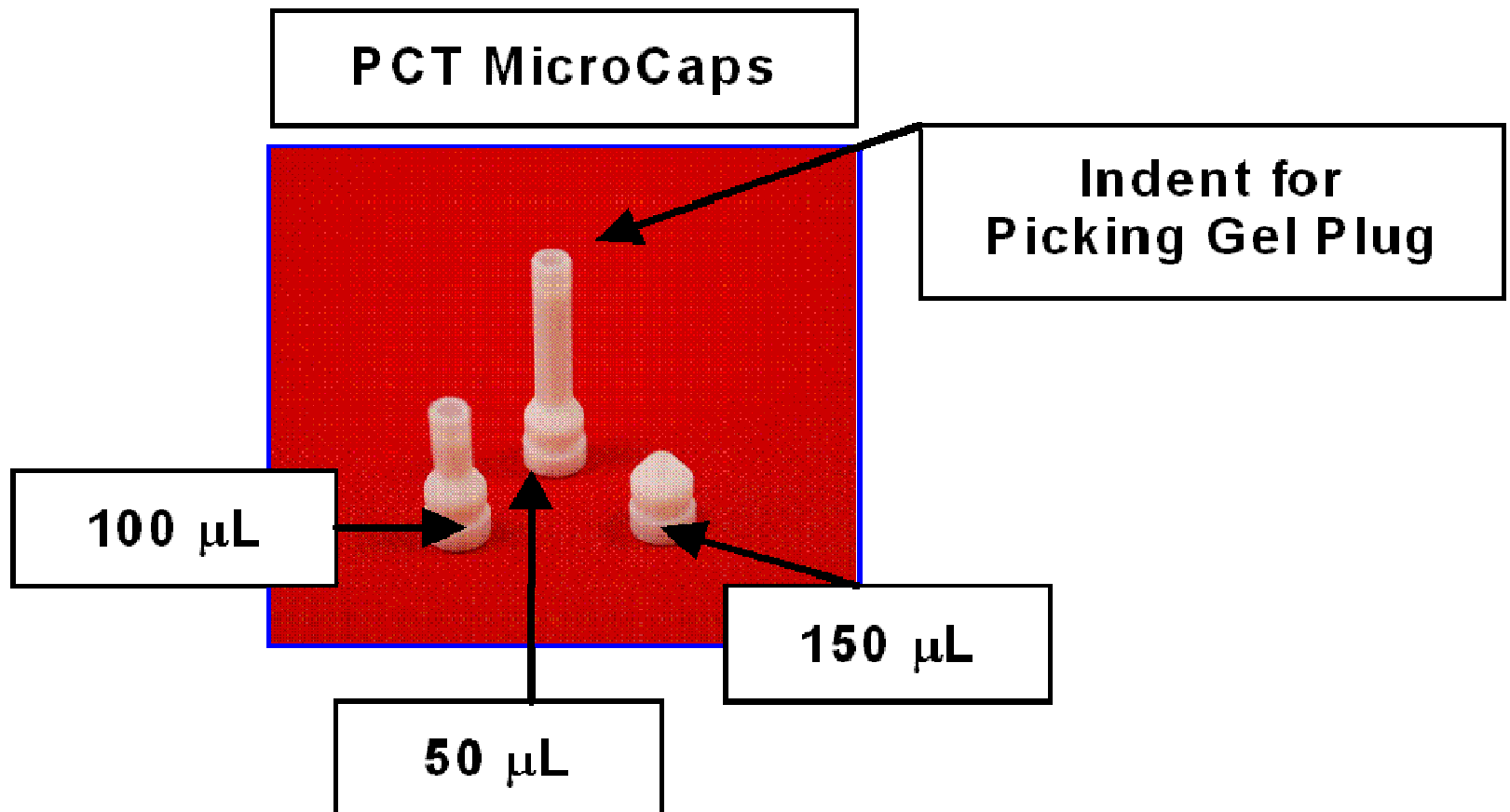


The PCT Shredder

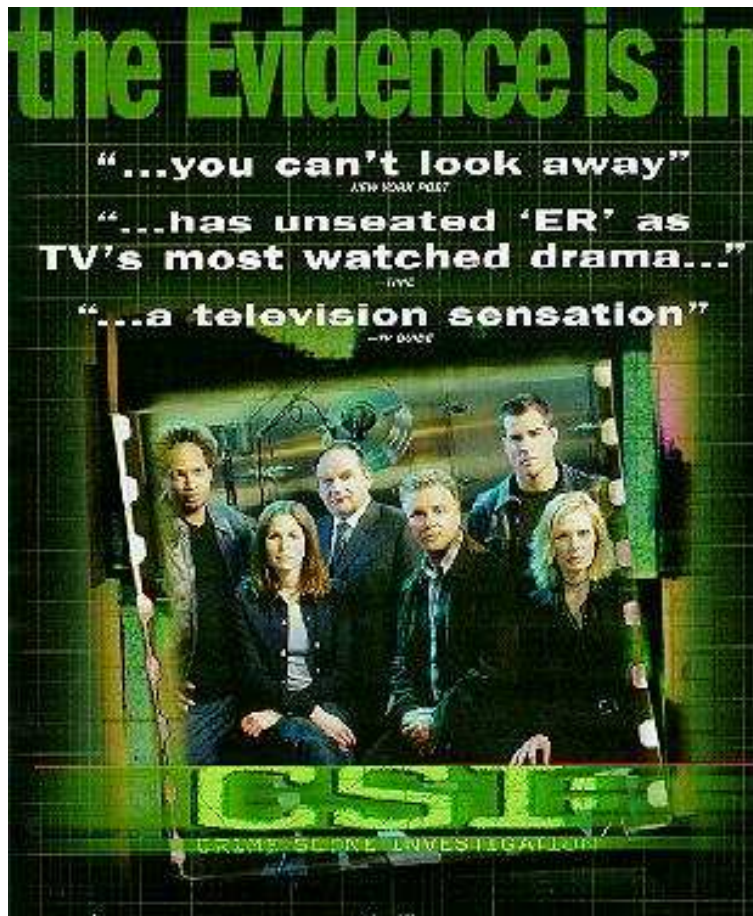
PCT MicroTube



PCT MicroCaps



ID in 60 Minutes



Forensic Programs Evaluating PCT

University of North Texas

Low Copy Number (Touch Samples, Swabs)

Marginal Quality Bone

MitoDNA from Hair

Dr. Bruce Budowle

Minnesota Department of Public Safety

Marginal Quality Bone

Low Copy Number (Touch Samples, Swabs)

Dr. Anne Gross

Florida International University (1)

Differential Lysis of Sperm and Vaginal Cells

Dr. Bruce McCord

Florida International University (2)

Vacuum Filtrates

Dr. Dee Mills

Pressure BioSciences, Inc.

Low Copy Number (Touch Samples, Swabs)

Dr. Alexander Lazarev

Dr. Vera Gross

**PBI is extremely grateful to Promega Corp.
and Mr. Len Goren (Global Director, Genetic Identity Promega Corp.)
for supporting the development of novel PCT-enhanced
forensic methods in these laboratories.**

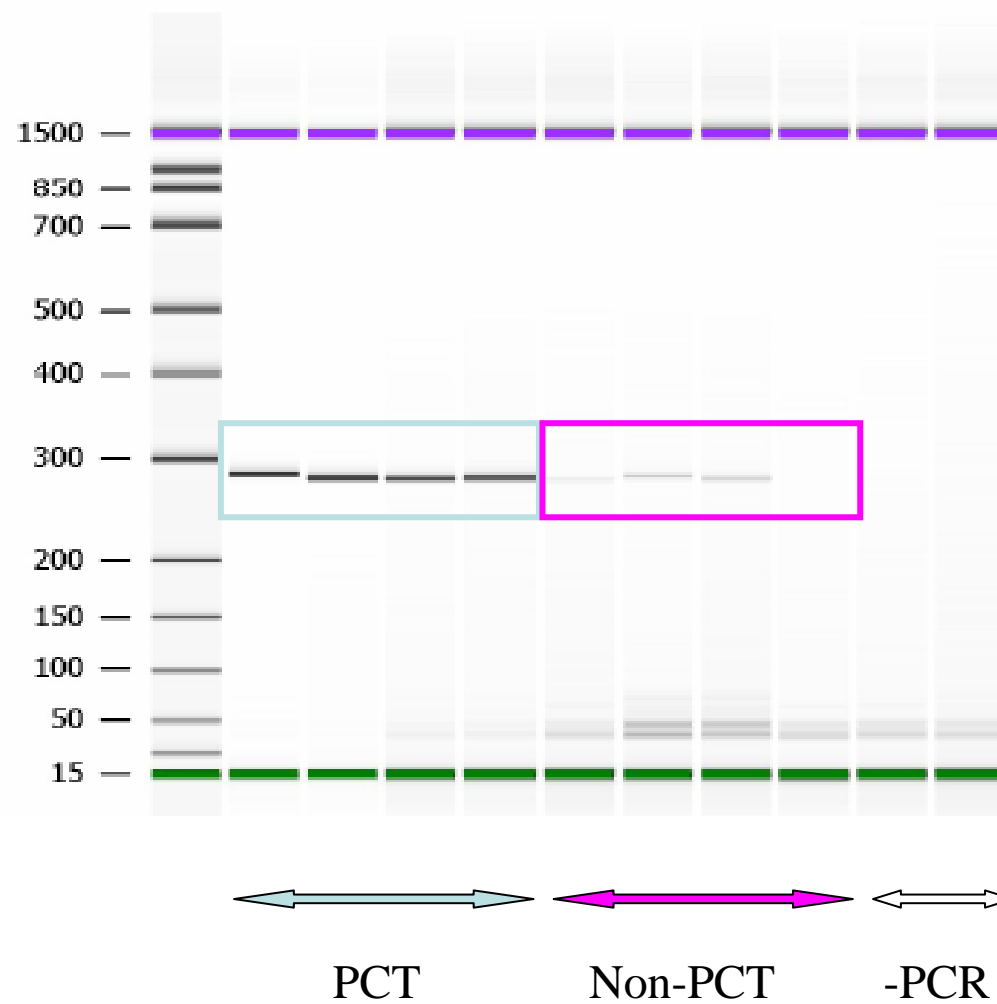
The logo for Pressure BioSciences, Inc. (PBI) features the letters 'PBI' in a stylized, bold font. The 'P' and 'B' are blue, while the 'I' is red. The letters are slightly shadowed and have a dynamic, italicized appearance.

In Sight

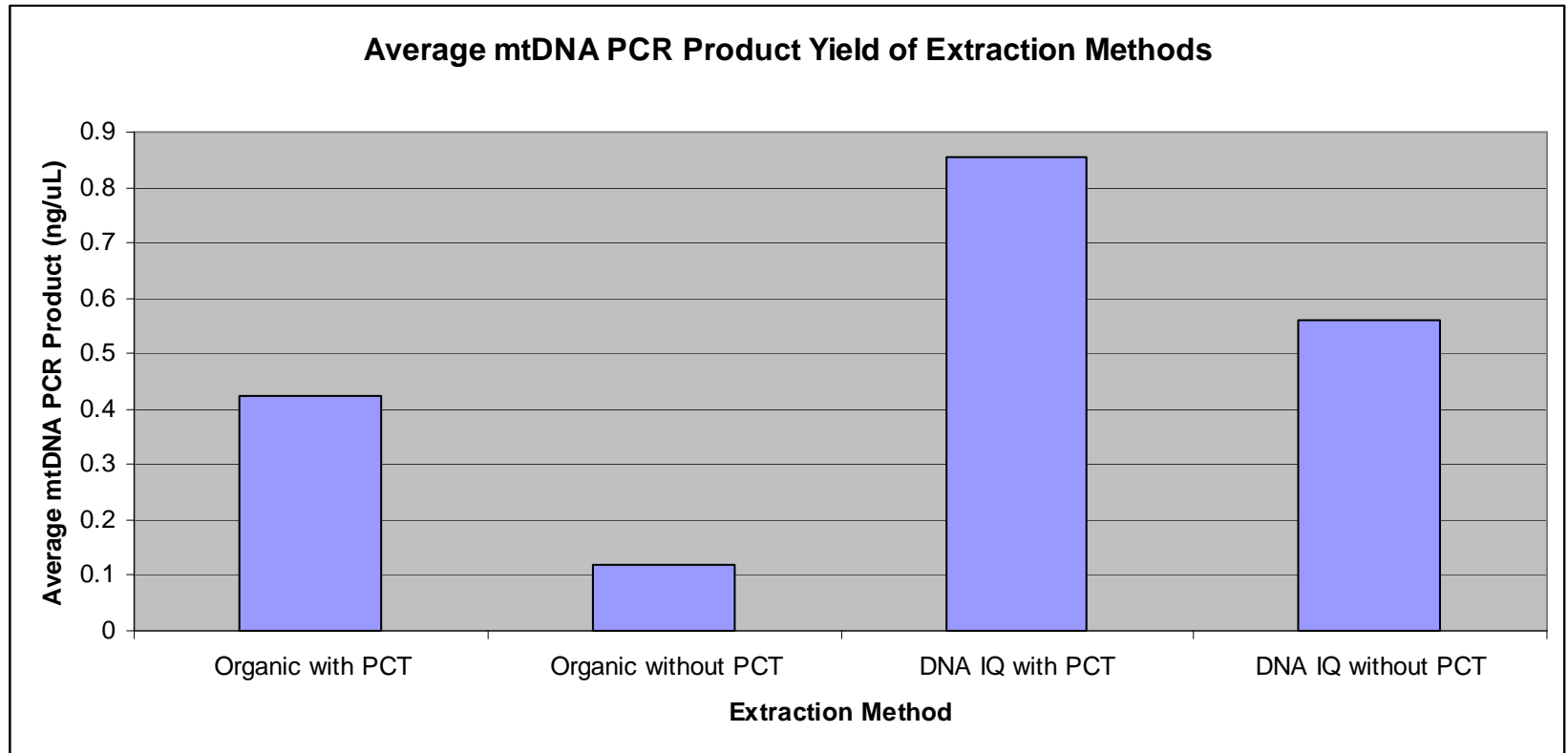
Again, the front-end sample preparation is perhaps the biggest hurdle to overcome, since crime-samples present themselves in myriad manners and these macro-samples may not be readily amenable to microfluidic processing.

Bruce Budowle and Angela van Daal

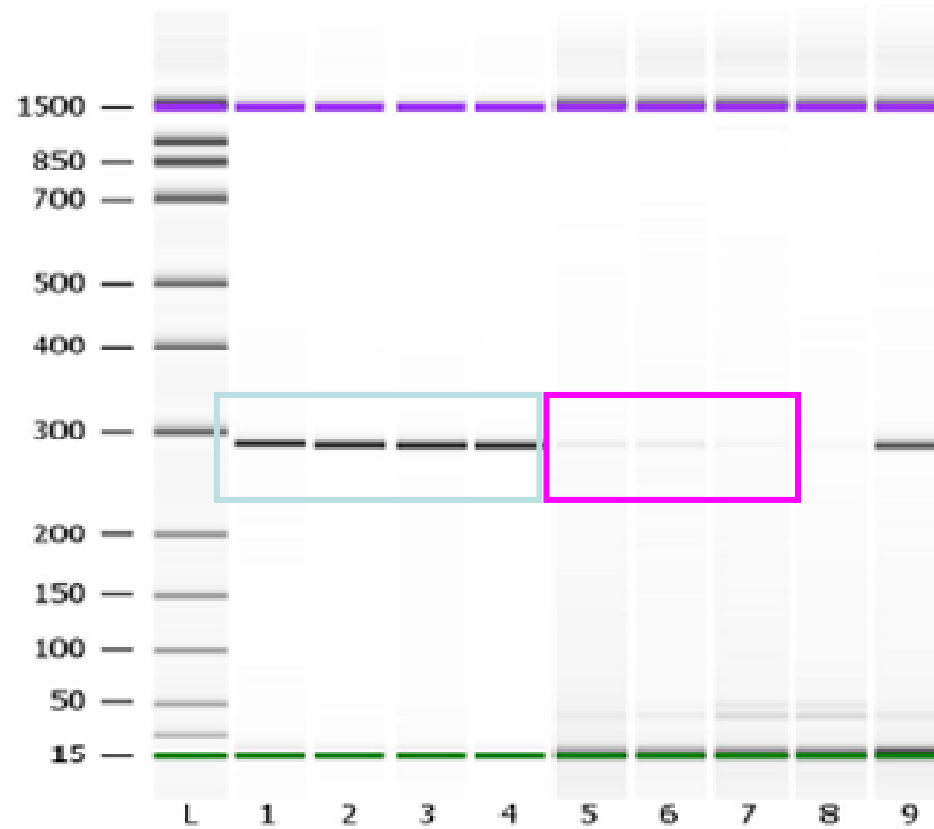
mtDNA from a Single Human Hair



Hair Samples

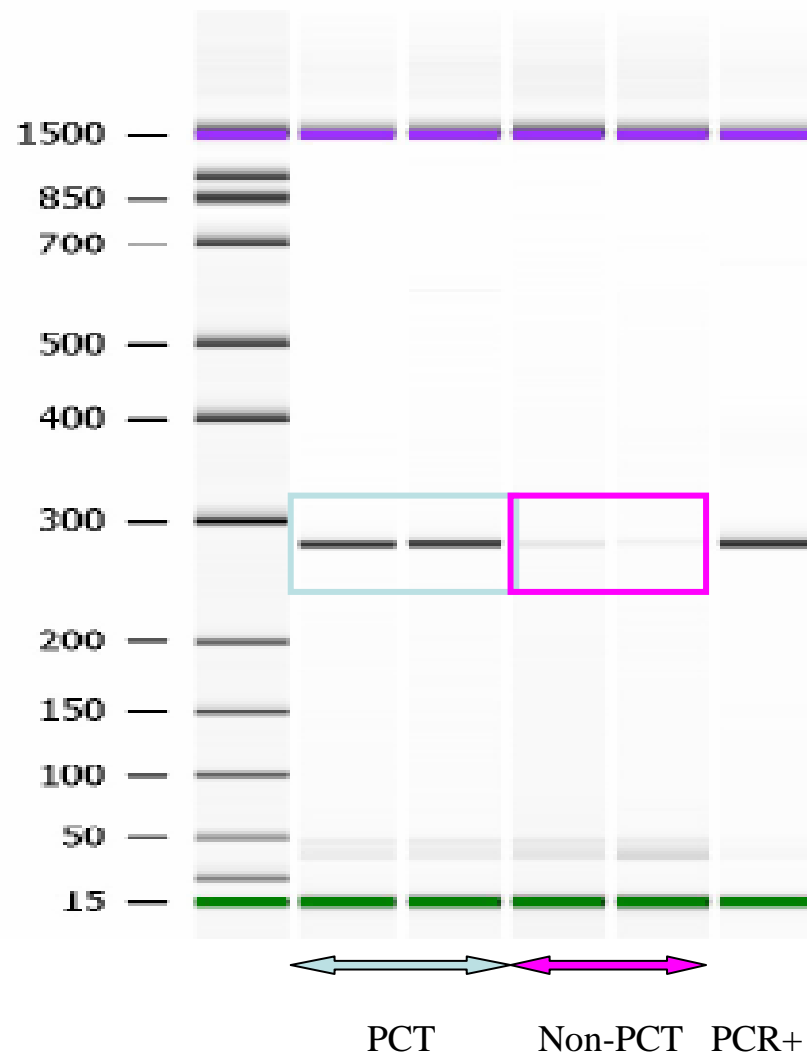


mtDNA from Human Skin Collected on Tape

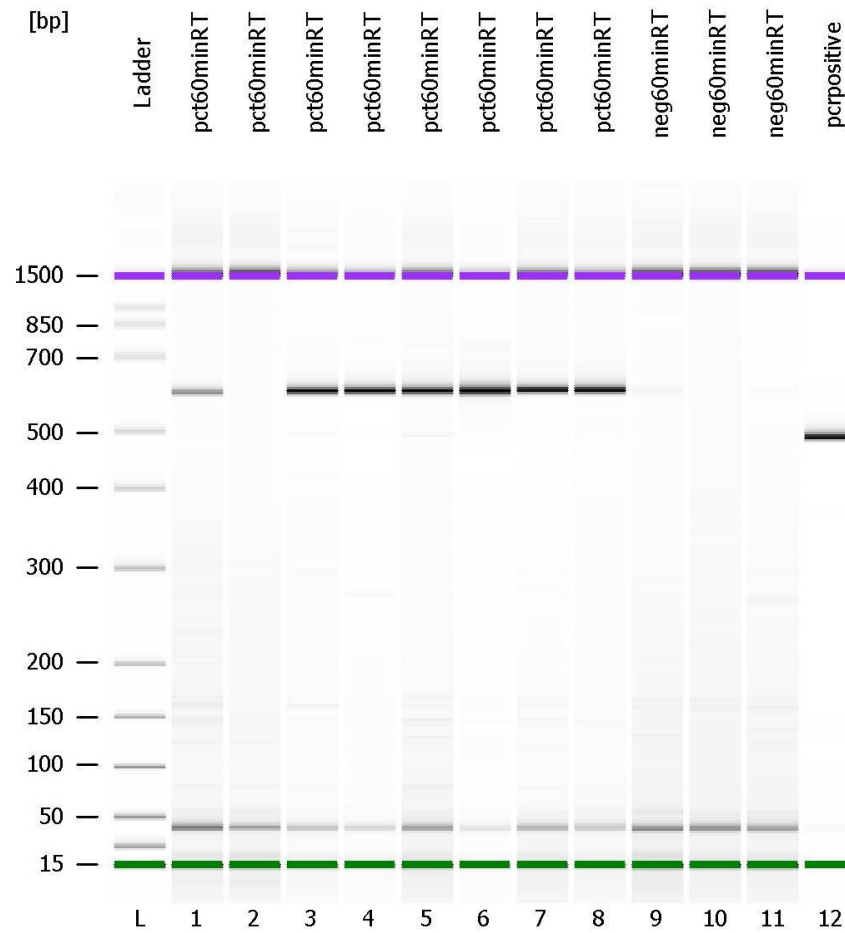


← PCT → ← Non-PCT → PCR
- +

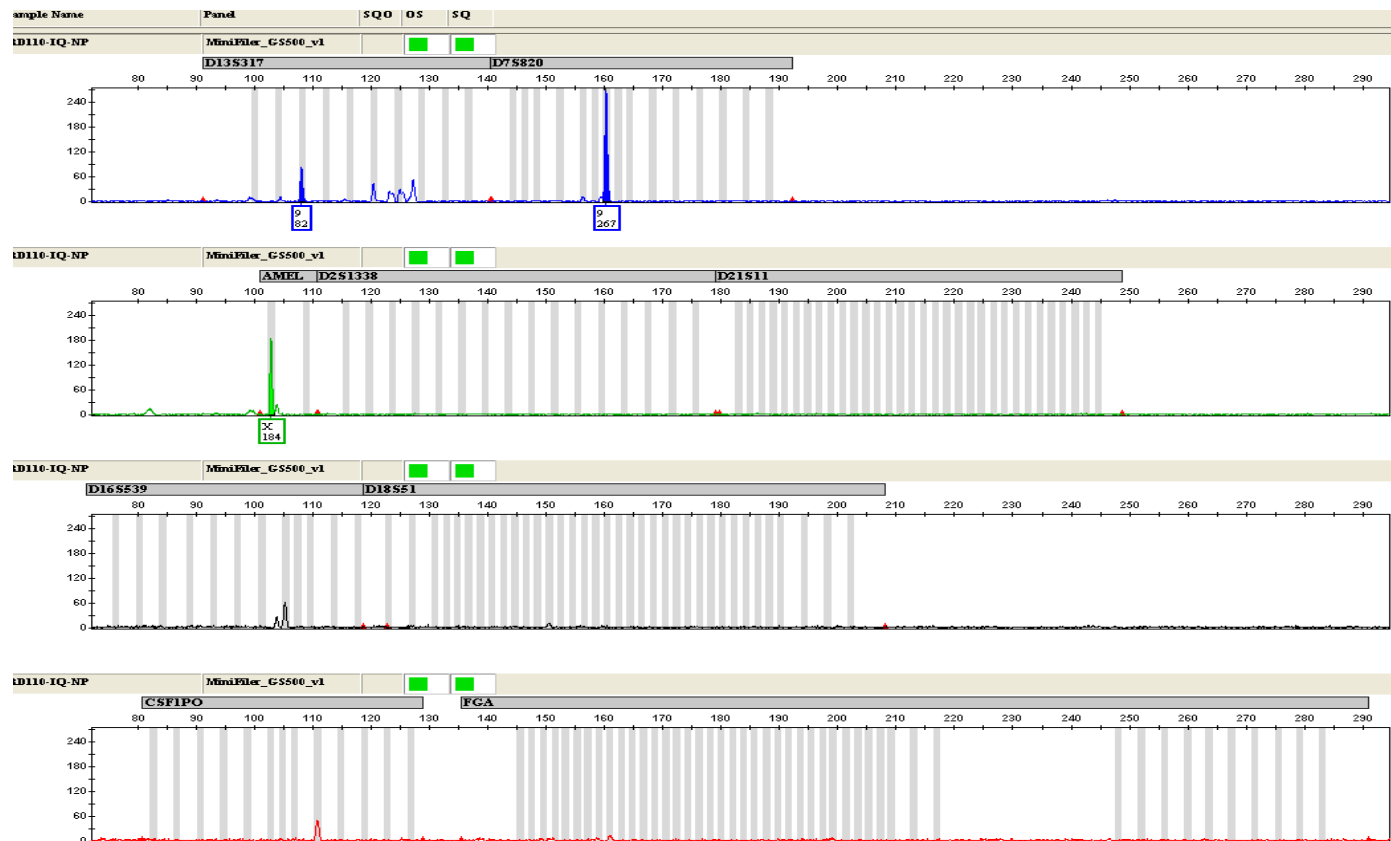
mtDNA from Human Blood from a Single Fiber



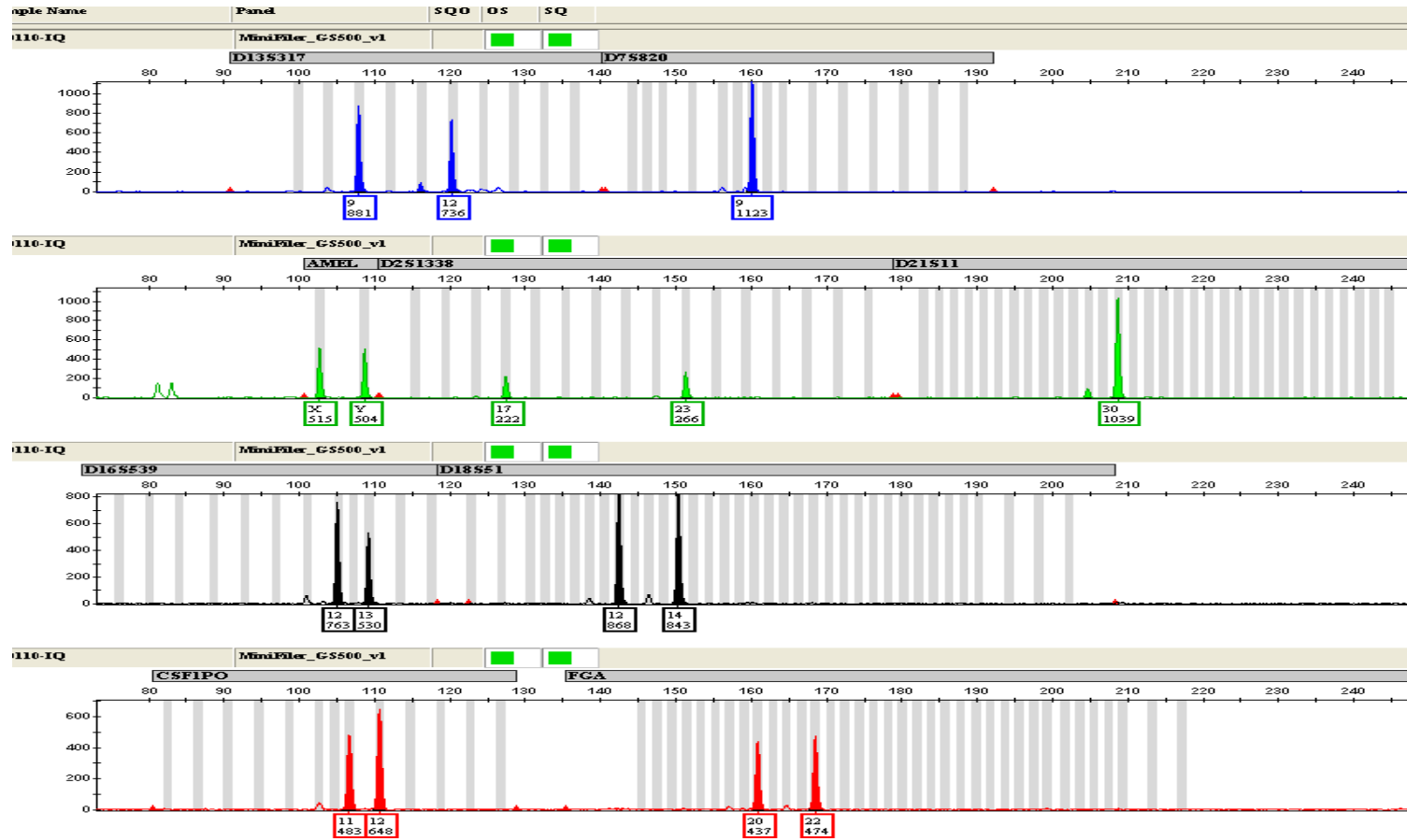
Genomic DNA from Pig Bone



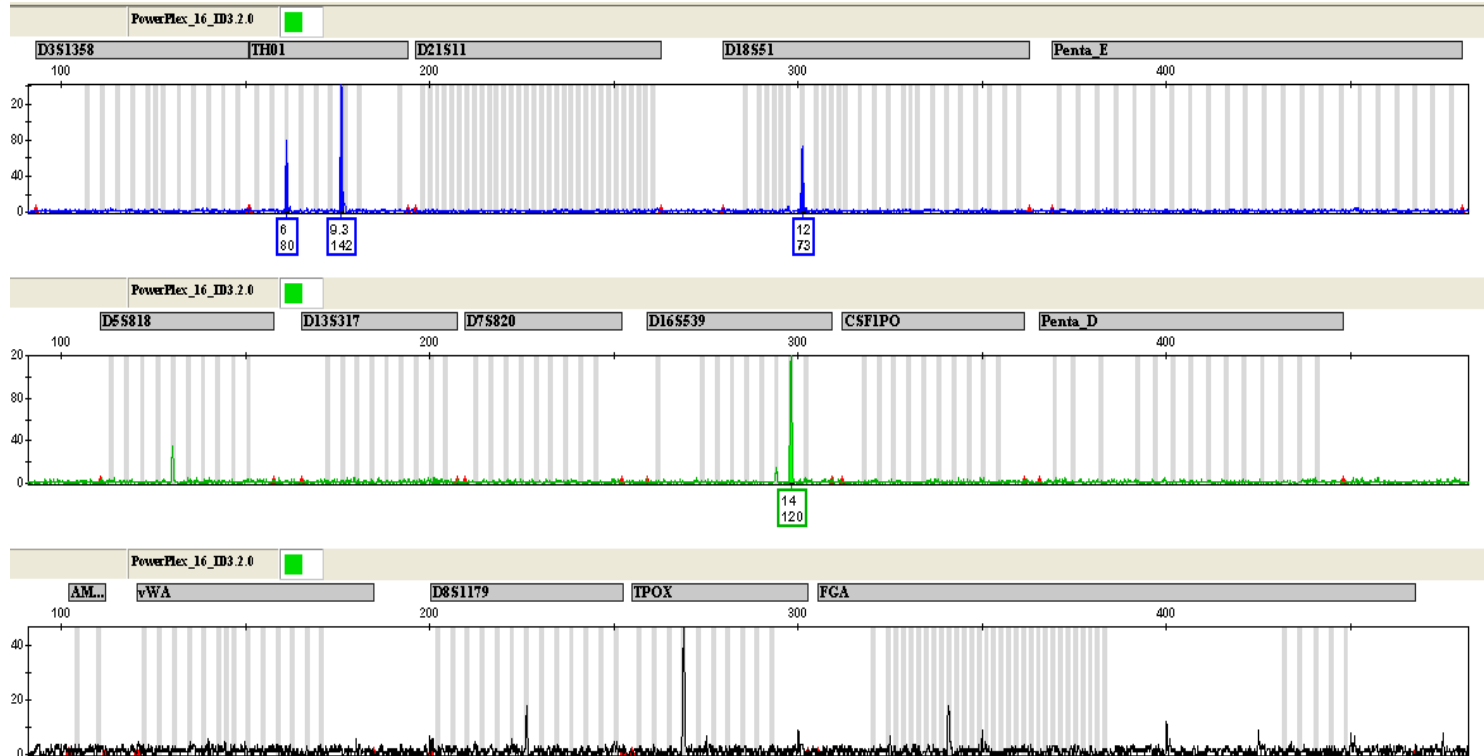
Bone #10: No Pressure



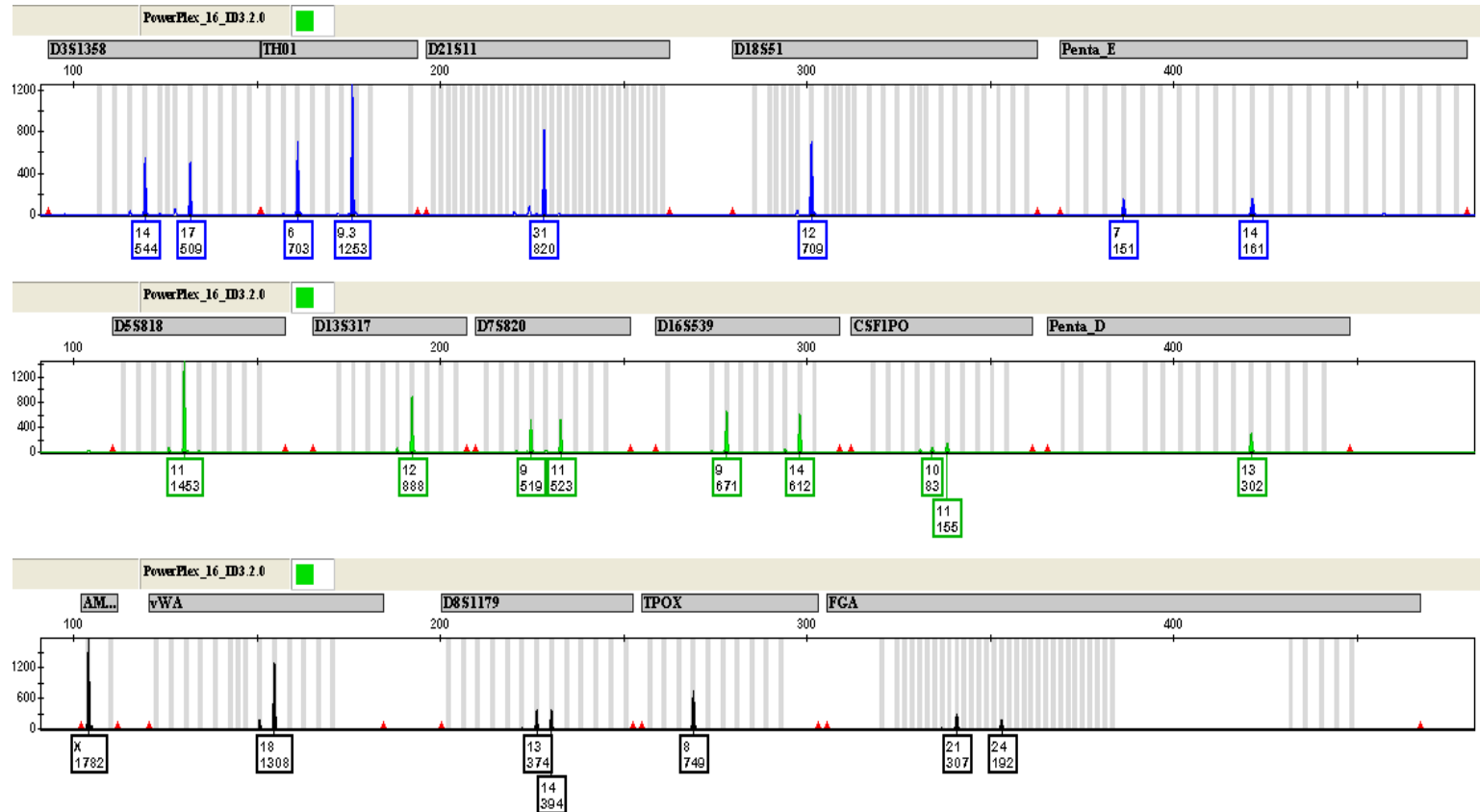
Bone #10: with PCT



Bone #4: No Pressure

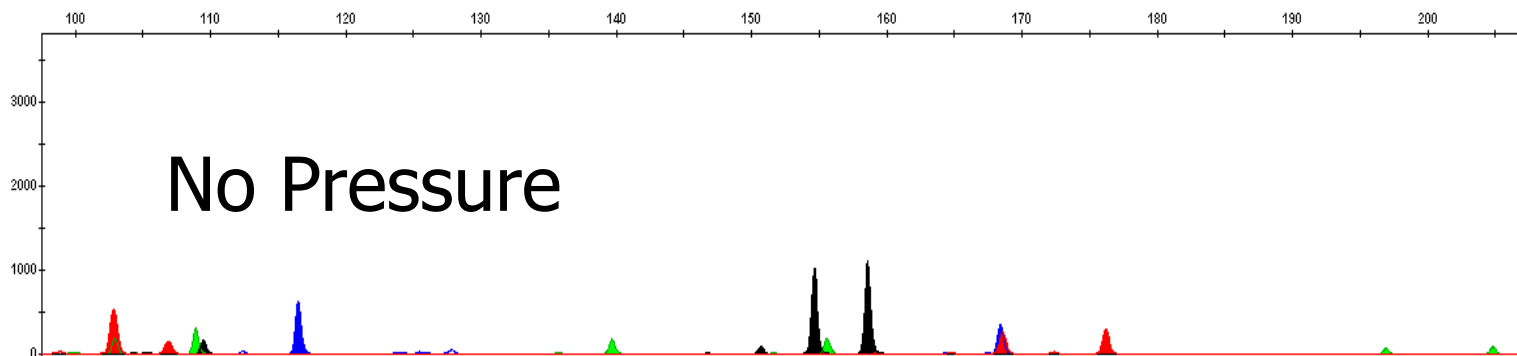


Bone #4: with PCT

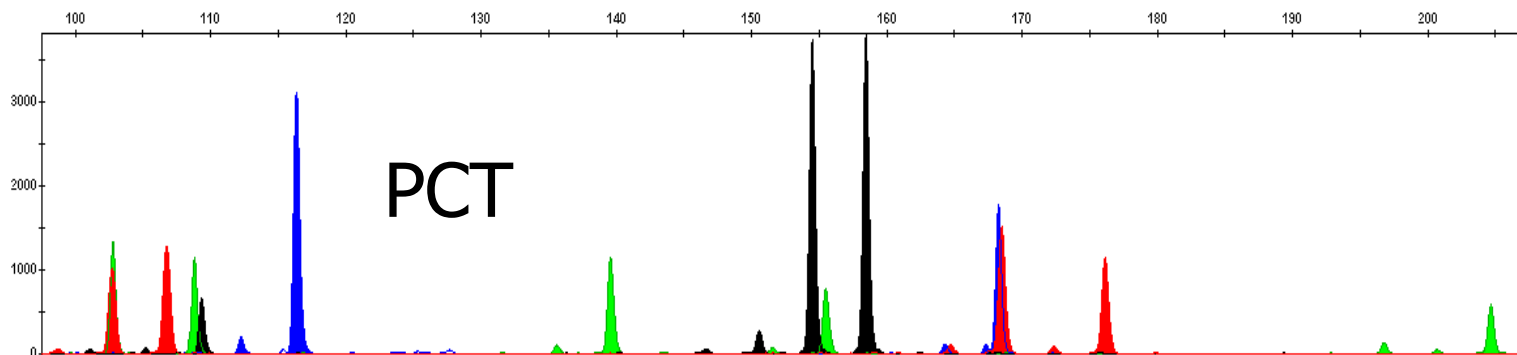


Bone #1

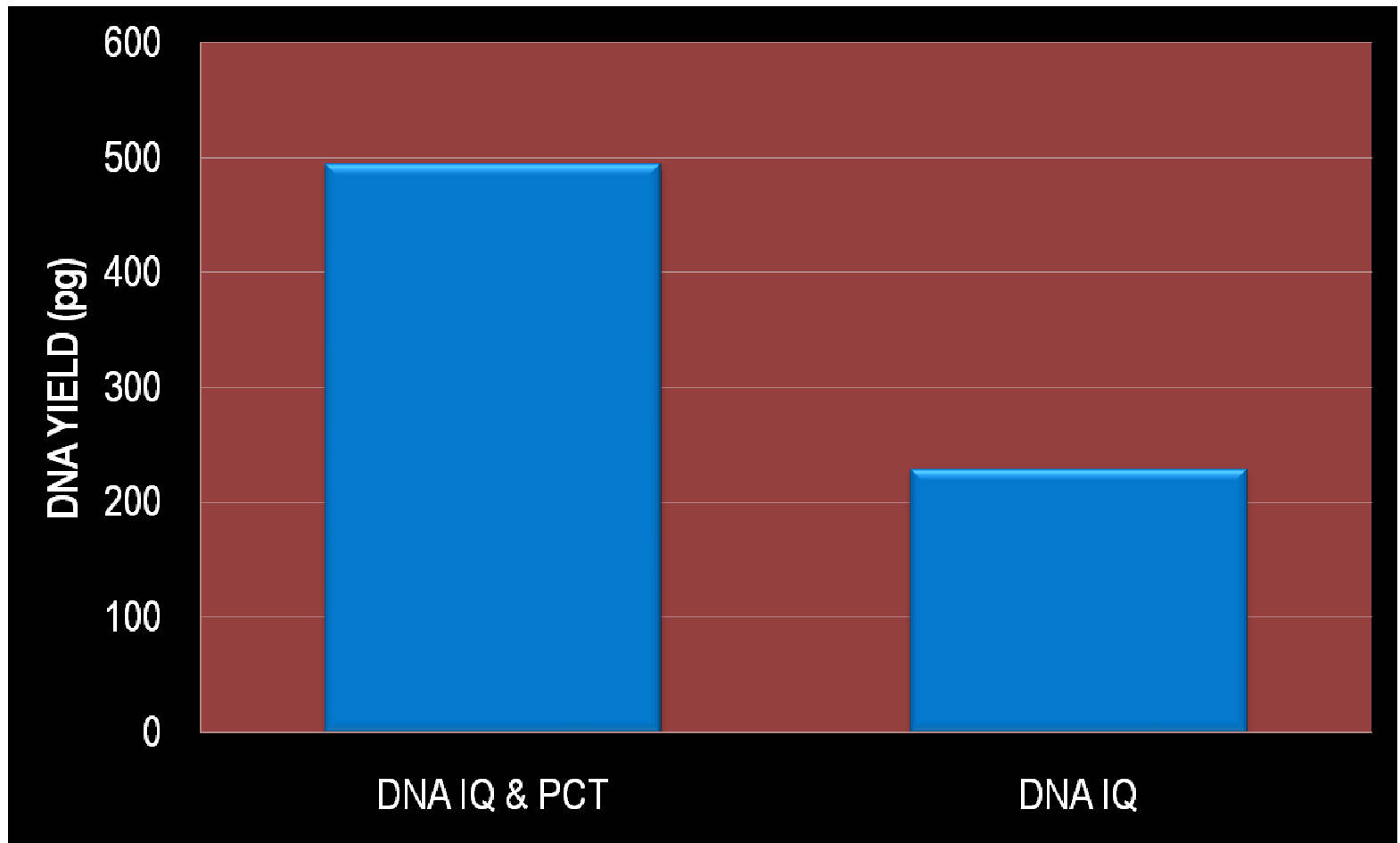
IQ-NP MiniFiler_GS500_v1 ■



IQ-PCT MiniFiler_GS500_v1 ■



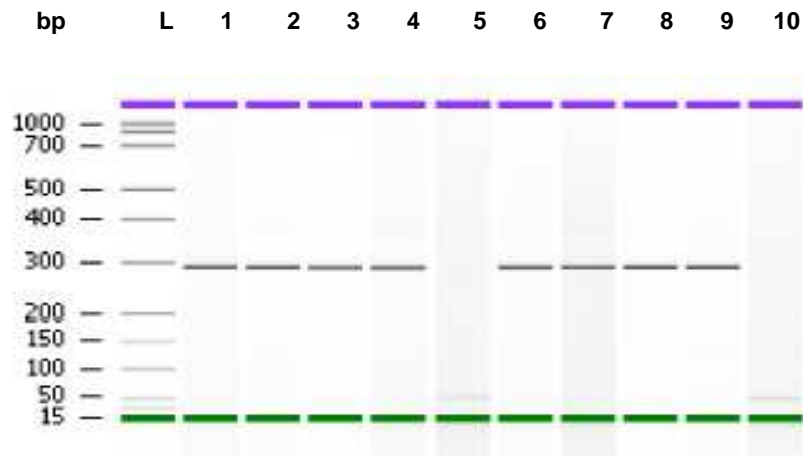
Average DNA Yields from Bone



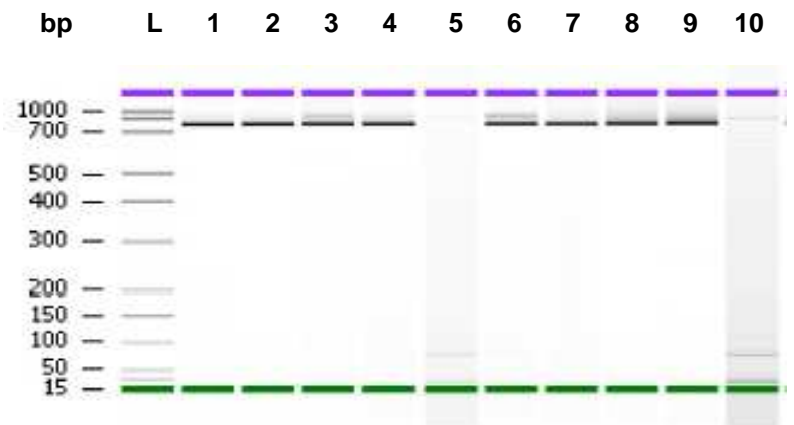
PBI

DNA Extraction from Human Bone Samples (Korean Samples)

Gel One: PCR products of mitochondrial gene amplification



Gel two: PCR products of human B-actin gene amplification



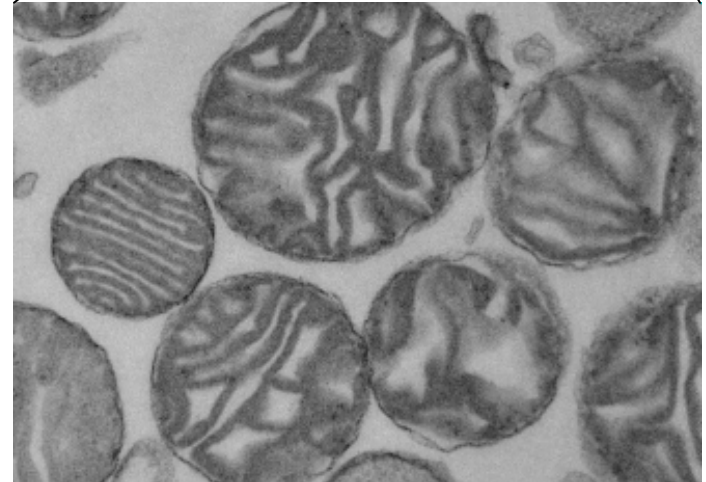
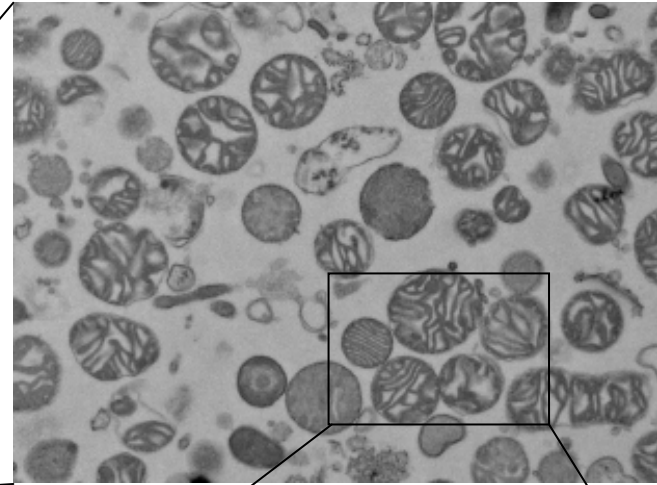
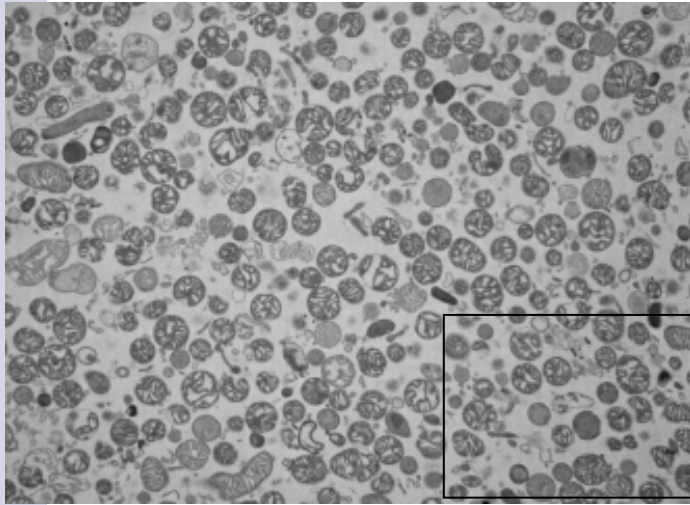
Differential Lysis of Sperm and Vaginal Cells

Differential Lysis: Exploiting The Pressure Profile

Variables

- Pressure
- Number of Cycles
- Buffer
- Temperature

“Live” Kidney Mitochondria Isolated by PCT



Summary

The PCT SPS combines the elements of most competing sample preparation technologies in one powerful package:

- **Compatible** with the **chemical disruption buffers** and downstream analysis buffers
- **Little or no grinding and shearing** that destroy macromolecules
- Precise **temperature control**, including **cryogenic** conditions
- **Uniform energy distribution** throughout the sample
- Precise control of **molecular disassembly**
- **Compatible** with a wide variety of **organic solvents**
- **No exposure to metal** parts – preserves fragile redox-active chemical species and prevents activation of metalloproteases