Forensic Testing Using Pressure Cycling Technology (PCT)

Midwestern Association of Forensic Scientists
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Thomas E. Strader, MS-Biotech

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Abstract

- The recent emergence of new, cutting-edge technologies have allowed forensics scientists to significantly improve DNA collection, extraction, separation, purification, and analysis. One new technology being evaluated by multiple forensics laboratories is pressure cycling technology, or PCT.

- Current methods used in forensics laboratories in rape kit testing include differential organic extraction, laser microdissection, and filtration. These methods can be time-consuming, cumbersome, and inefficient.

- Studies performed in Dr. Bruce McCord's lab at FIU have shown that when special reducing agents are used in combination with precise pressurization, DNA from sperm can be selectively released and enriched from the mixture, leaving behind largely intact epithelial cells with the DNA remaining within the cell.

- The University of North Texas reported that touch-samples, which typically yield too little DNA for accurate typing, may be suitable for routine analysis if sample preparation incorporates ultra-high pressure methods such as PCT, as such methods have been shown to provide effective extraction and enrichment of DNA from the sample.

- The progress made by these two laboratories using PCT includes data showing faster time to result, automation, batch-processing up to 48 samples, and methods that may lead to standardized processes and protocols that have the potential to relieve the back-log of rape kit samples and better identify DNA from low copy number specimens.

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Overview

New technology is available to enhance the safety, efficiency, and quality of DNA testing in numerous areas including:

- Sexual assault
- Degraded bone
- Touch sample cases

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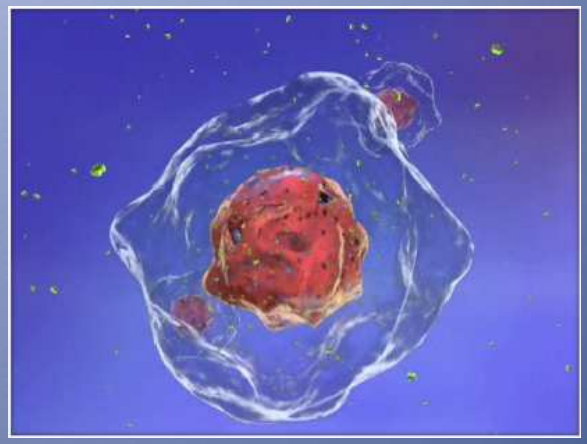
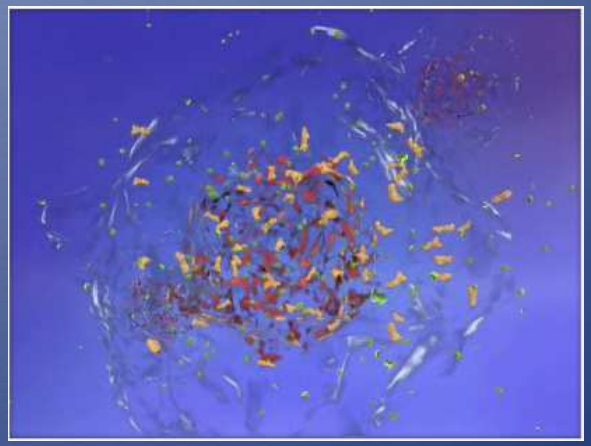
PCT Technology Overview

- In closed container, PCT applies and releases pressure on the sample from ambient to 35,000 psi in user customizable intervals
- PCT uses hydrostatic compression differential of various cellular components to disrupt cell with gentle but highly powerful forces



Intact Cell



Cell Lysis with PCT

Image courtesy of: http://www.vet.purdue.edu/cpr/secondaryinjury3.html

video

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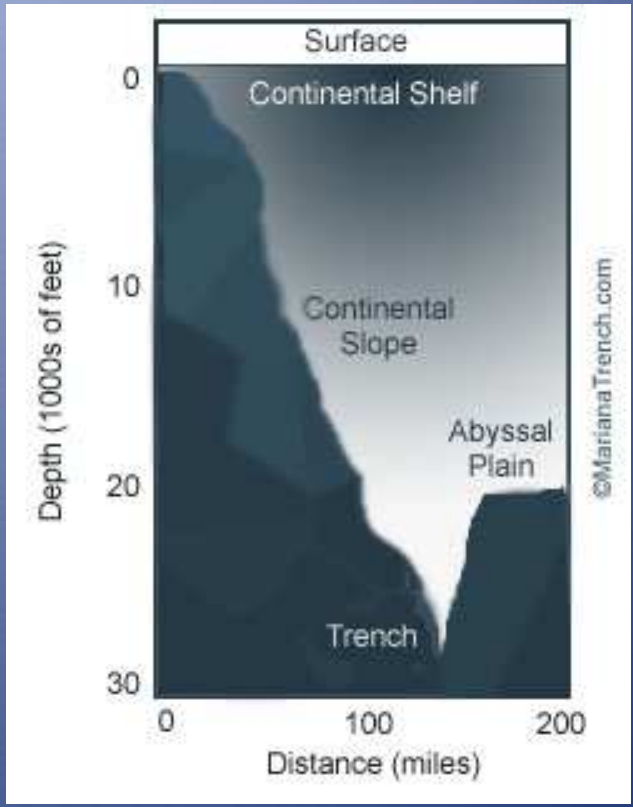
PCT Technology Overview

- H_20 is non-compressible at atmospheric or near atmospheric pressure
- H_20 is 7-8% compressible at 35,000 psi
- For reference, the bottom of the Mariana Trench is 11,033 meters with a pressure of 15, 750 psi or about 8 tons per square inch



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The Barocycler

- Bench top instrument
- Optional heating/cooling
- Uses a programmable logic controller
- Processes up to three samples simultaneously

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The 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

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PCT Facts

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PCT Facts

- PCT alters conformations and interactions of biomolecules
- Destabilizes secondary structures
- Does not denature or inhibit enzymes
- Does not appear to degrade DNA below levels practical for forensic analyses

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PCT Increases Yield for Bone and Hair

Pressure Cycling Technology (PCT) Applications for DNA Extractions from Challenging Forensic Samples

Suzanne Gonzalez, Elizabeth Feller, Dixie Peters, Bruce Budowle, and Arthur Eisenberg
University of North Texas Health Science Center, Institute of Investigative Genetics,
UNT Center for Human Identification, 3500 Camp Bowie Blvd., Fort Worth, TX 76107

20TH INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION
THE REAL DEAL
LAS VEGAS, NEVADA · OCTOBER 12-15, 2009

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DNA IQ Isolate and Quantitate

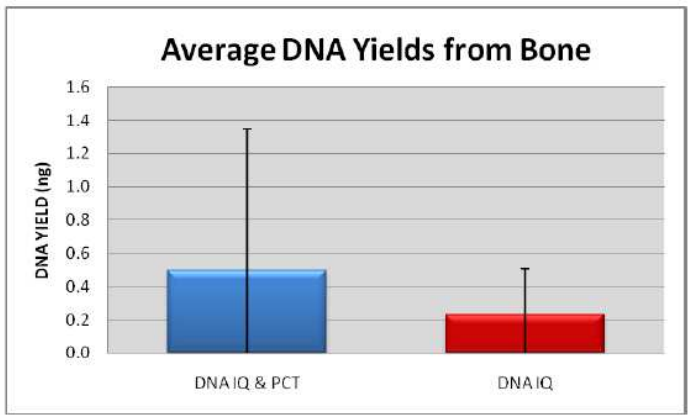

Figure 1. **Average DNA Yields from Bone**. The average DNA yields were calculated for 12 challenging bone samples subjected to Pressure Cycling Technology and DNA extraction/purification using the DNA IQ™ Casework Sample Kit for Maxwell® 16 (DNA IQ & PCT) and compared to average yield of duplicate samples processed with the DNA IQ™ Casework Sample Kit for Maxwell® 16 without PCT (DNA IQ). Samples processed with PCT resulted in at least a two-fold increase in DNA recovery.

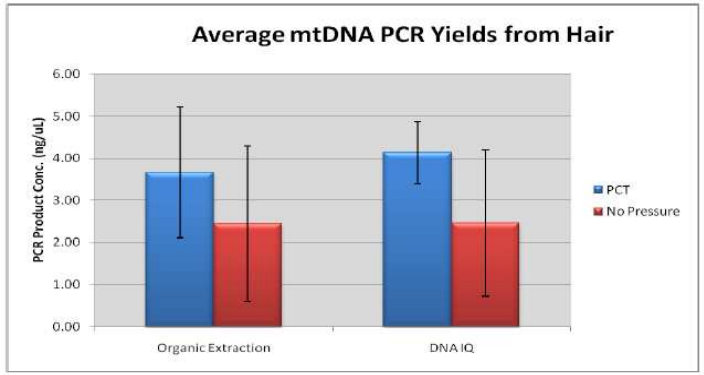

Figure 2. **Average mtDNA PCR Product Yields from Hair**. The average PCR product yields for mitochondrial DNA hypervariable region 1 (HV1) were calculated for single 2cm hair shaft cuttings sampled from 4. Samples were extracted using both an organic method (Organic Extraction) or extracted using the DNA IQ™ Casework Sample Kit for Maxwell® 16 (DNA IQ). Average yields were compared for each method run in combination with Pressure Cycling Technology (PCT) or without PCT (No Pressure). Samples processed with PCT resulted in a 67% increase in amplified product for samples extracted with organic method. An average increase of 59% in product yield was obtained using the DNA IQ™ Casework Sample Kit for Maxwell® 16 and PCT compared to the no pressure control samples.

http://www.promega.com/geneticidproc/ussymp20proc/oralpresentations/Gonzalez.pdf

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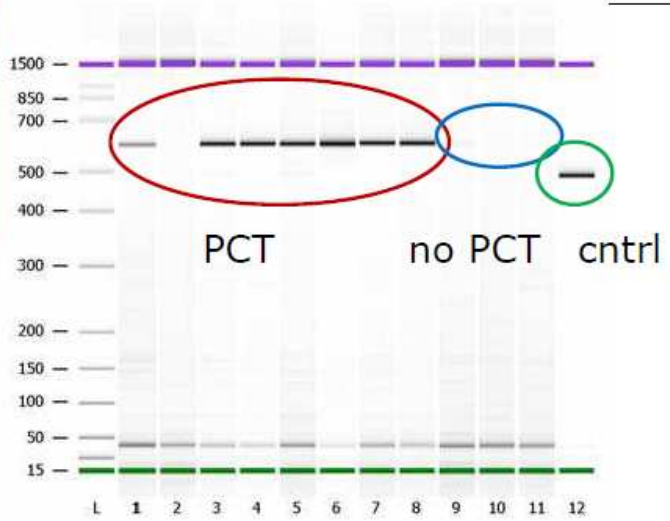
What has been achieved thus far?

DNA from Bone

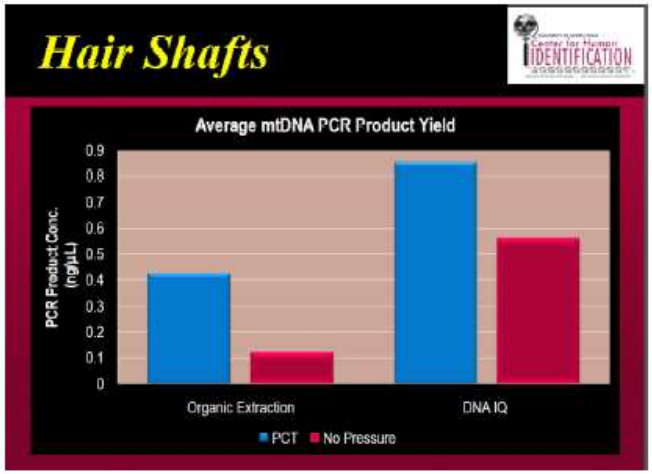


PCR products from pig bone extracts

Lanes 1-8 bone incubated with acetic acid/EDTA for 60 min and followed by 10 cycles of PCT at 4°C.

Lanes 9-11 were incubated with acetic acid for 1 hr but no PCT

DNA from hair



PCR products from hair

UNT data – organic vs DNA IQ w/ and w/out pressure

Note that the pressure treatment does not interfere with enzymatic digestion

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PRESSURE CYCLING TECHNOLOGY (PCT): APPLICATIONS FOR FORENSIC DNA ANALYSIS

Pam Marshall, MS

Research Appreciation Day, 2010

UNIVERSITY OF NORTH TEXAS Center for Human IDENTIFICATION

Laboratory of Forensic Anthropology • Laboratory for Molecular Identification

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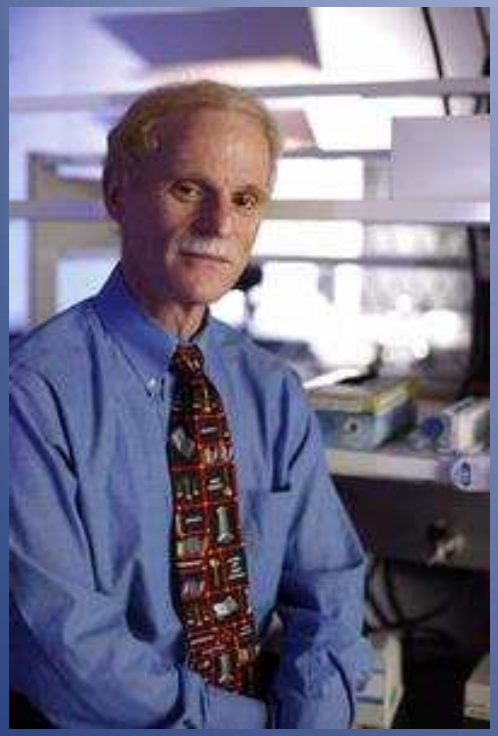
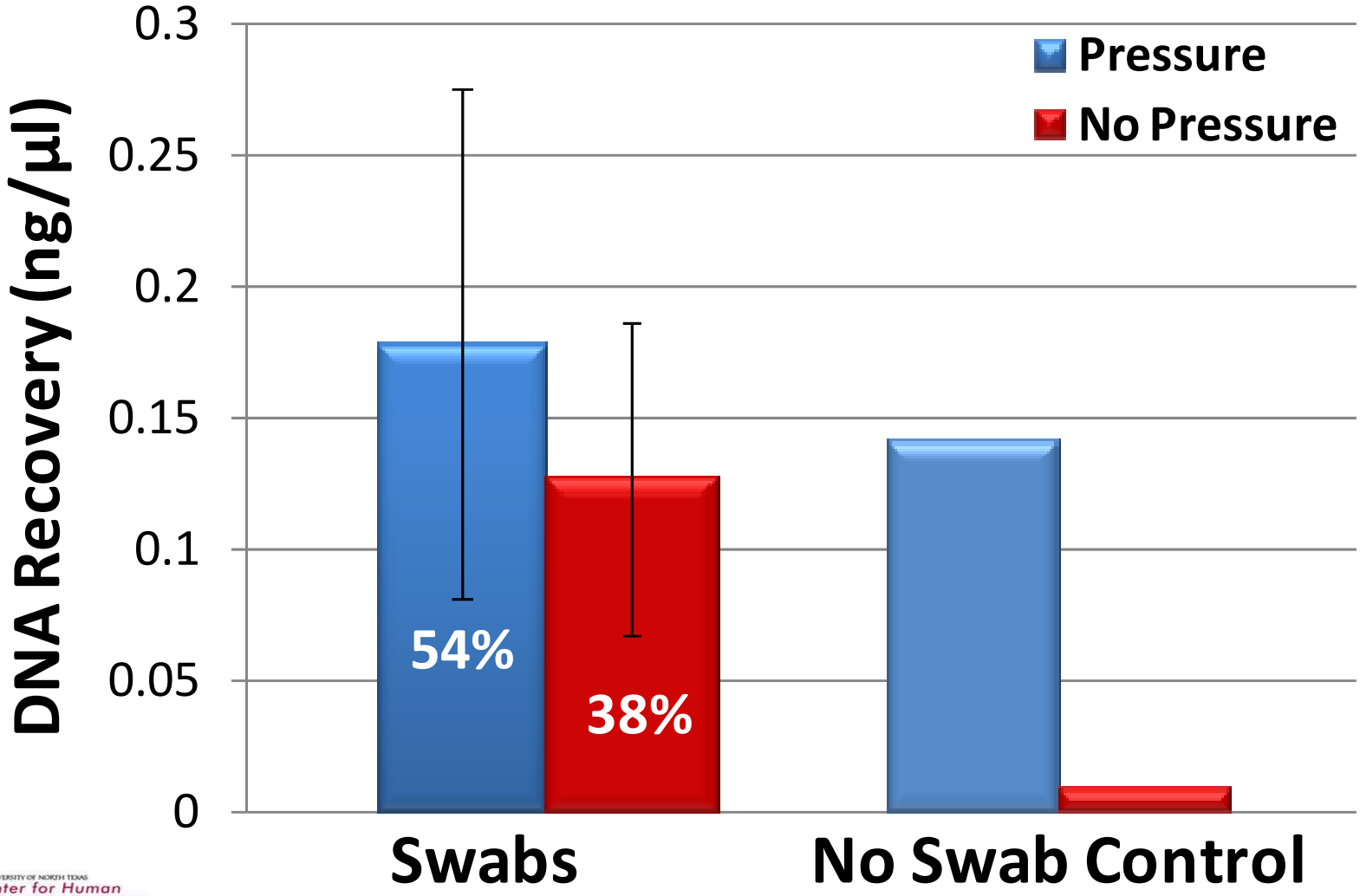
Bruce Budowle



"With this (Pressure BioSciences) technology, we've been able to get DNA from samples that yielded no results before."

http://www.youtube.com/watch?v=x6zDnMnqIzI

Pressure vs. No Pressure (50 cells/µl)



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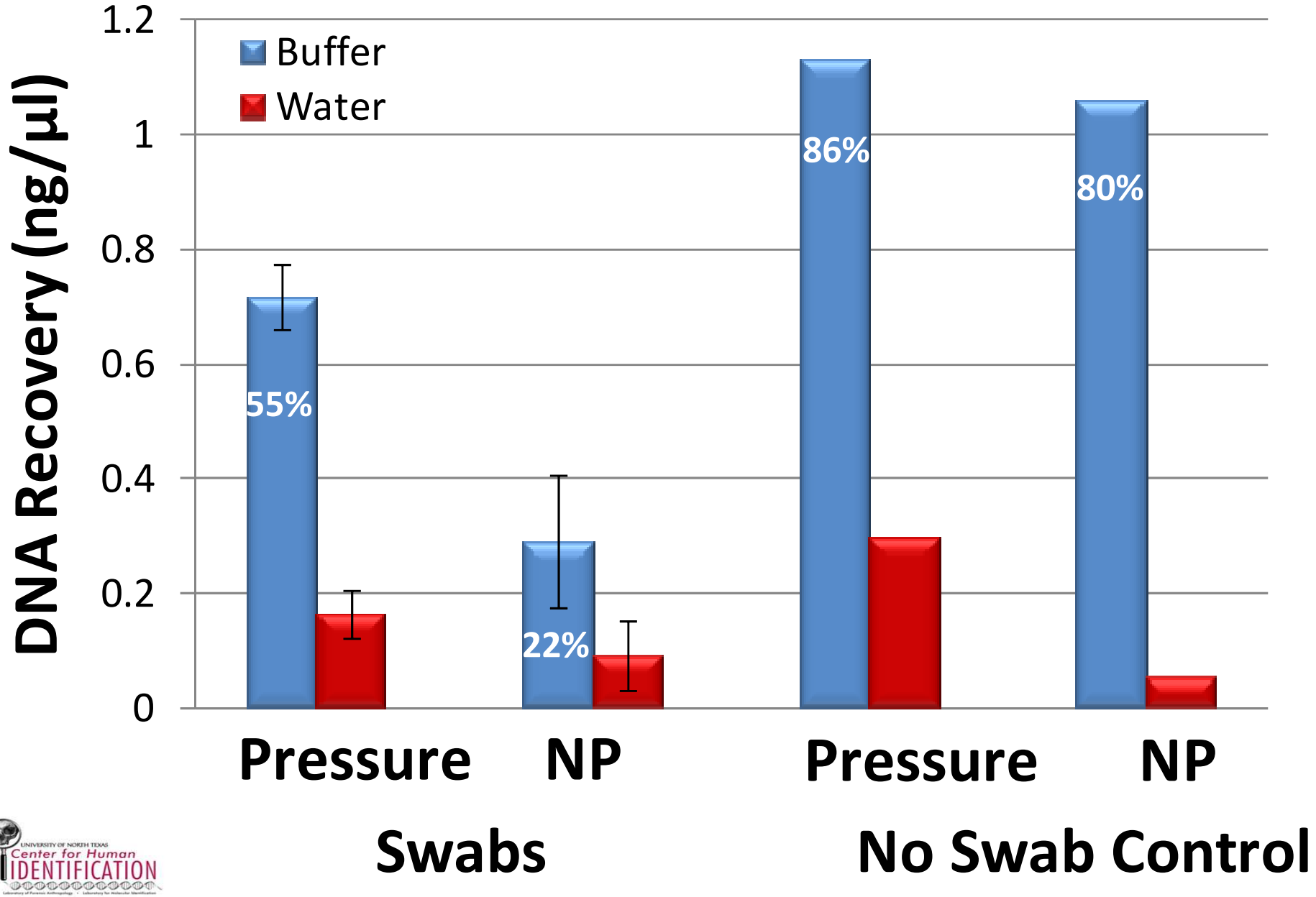
Buffer vs. Water



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Results Summary

- The data illustrate increased DNA yield in samples following PCT compared with those samples not exposed to pressure technology.
- These results indicate that PCT is a viable method to enhance DNA recovery from forensic samples.
- PCT can be used in conjunction with commercially available extraction reagents.

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Conclusions

- This research study demonstrates the capabilities and potential of PCT applications for enhancing robustness of forensic DNA analysis of biological evidentiary samples.
- The impact is that some samples that traditionally yield too little DNA for typing may now be suitable for routine analysis.
- More cases may be solved with this combined approach of PCT and DNA extraction.

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DNA recovery by pressure cycling and its potential application to differential extraction

Deepthi Nori and Bruce R. McCord*
Department of Chemistry and Biochemistry
Florida International University
mccordb@fiu.edu
www.fiu.edu/~mccordb

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Sexual Assault Testing

- After a sexual assault is reported, physical evidence is collected from the victim and sent to the laboratory for testing.
- Evidence, such as DNA, that is in queue to be tested is defined as the backlog.
- Forensic laboratory resource and staffing levels have not increased with increase in DNA testing requests

ENDTHEBACKLOG

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What are the alternatives?

1. Continue status quo (unacceptable)
2. Increase resources and staffing levels to be able to handle case-load in a timely manner
3. Find new ways to streamline and automate testing protocols so more samples can be processed with the same staffing and modest increases in resources

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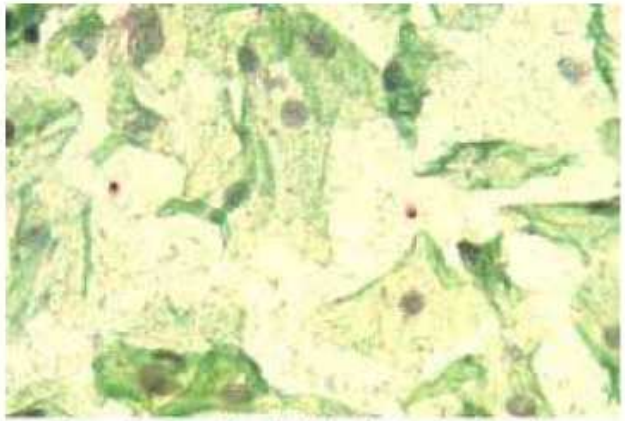
What problems exist with differential extraction?

1. The manual procedures are cumbersome and time consuming.
2. Sperm cells may lyse during initial digestion.
3. Female cells may not completely digest and contaminate sperm pellet



Sperm heads and epithelial cells as viewed under a high powered microscope using Christmas tree stain

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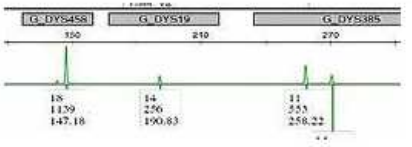
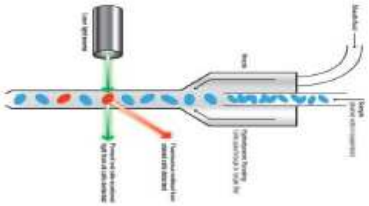
Alternatives to differential extraction

- Y STR typing



- Laser microdissection

- Cell sorting via microdevices or flow cytometry



- Pressure cycling

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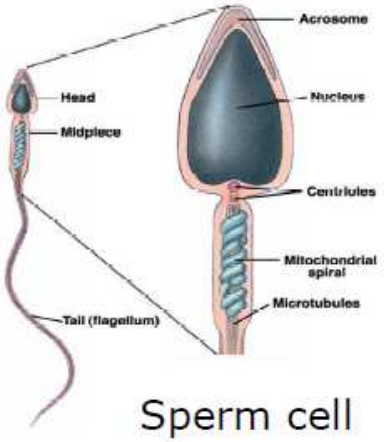
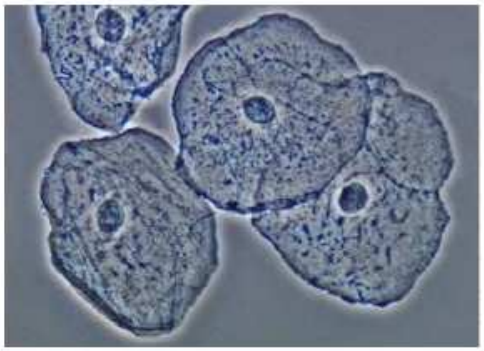
Our hypothesis:

- Sperm and epithelial cells should respond differently to pressure cycling based on their different composition
  - Epithelial cells are larger, with more diffuse structures. They should be more distorted by pressure, and thus more sensitive to its effects.
  - Sperm DNA is associated with protamines, proteins with a high cysteine content, crosslinked with disulfide bridges– dense packing of DNA (12-18% cysteine)
  - Epithelial cell nuclei are surrounded by histone proteins. These are not as cross linked as protamines – less dense packing (0.2% cysteine)



Sperm cell



Buccal Epithelial cell

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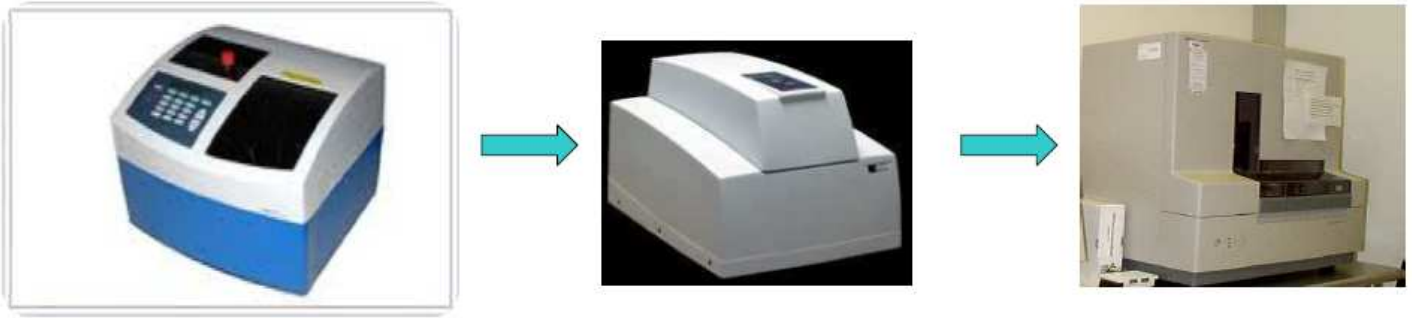
Experimental design

- Explore methods to preferentially lyse cells – leaving behind intact cells of specific type
- Alter physical parameters – pulse pressure, cycle number, temperature and time
- Utilize differences in buffer content – detergent, enzymes, DTT
- Monitor differential amplification by real time PCR and multiplex STR amplification



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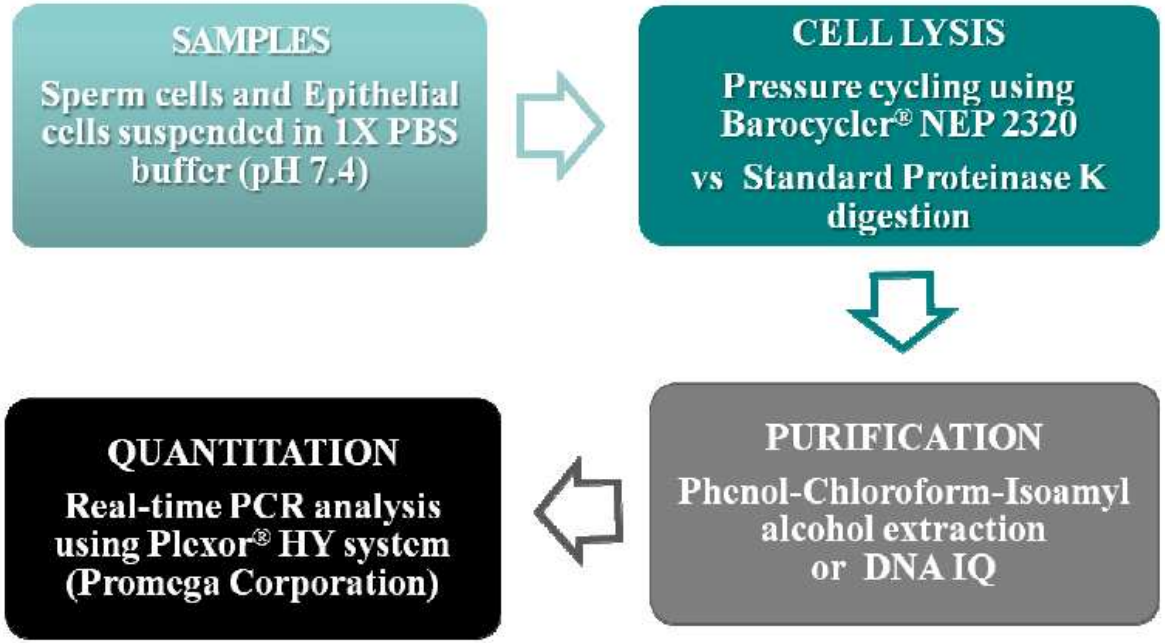
Experimental design

Key issue: controlling cell quantities and recovery of DNA



Use of real time PCR and extraction controls to provide confidence in results.

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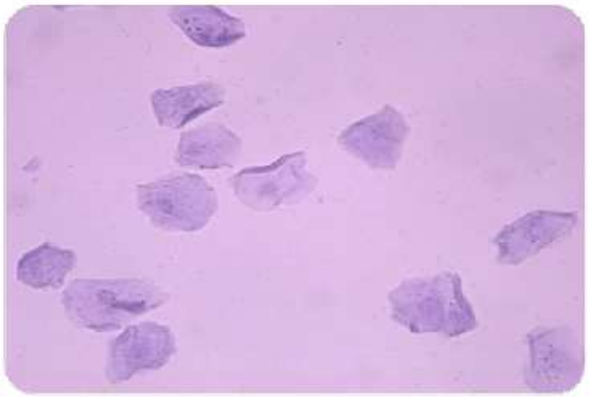
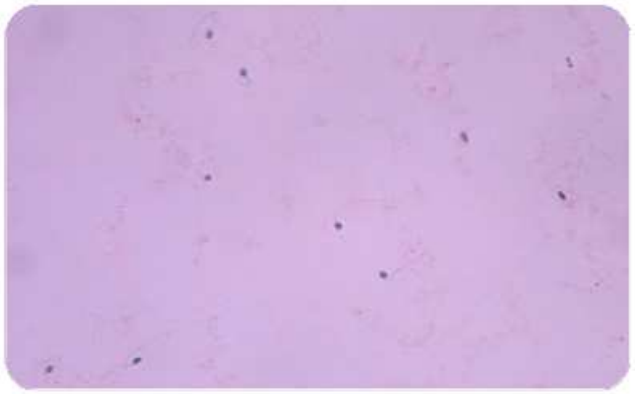
Initial PCT- Microscopic studies

Cell Visualization in PBS

- **Cells stained with 0.4% Trypan blue (dye exclusion method) following Pressure treatment**
- **Color indicates PCT treatment is causing take-up of dye**



Vaginal epithelial cells



Sperm cells

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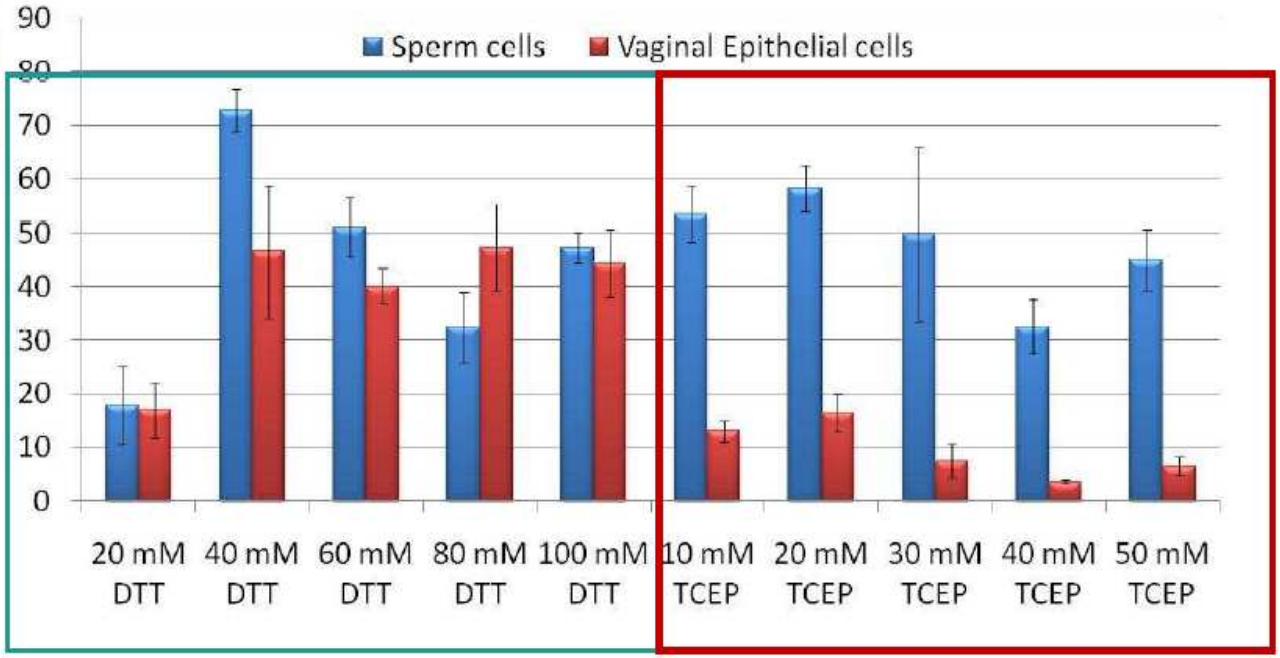
A comparison: DTT vs TCEP

Switching to **TCEP** caused an increase in selectivity between sperm cell and epithelial cell lysis



Dithiothreitol (DTT)

Tris (2-carboxyethyl)phosphine (TCEP)

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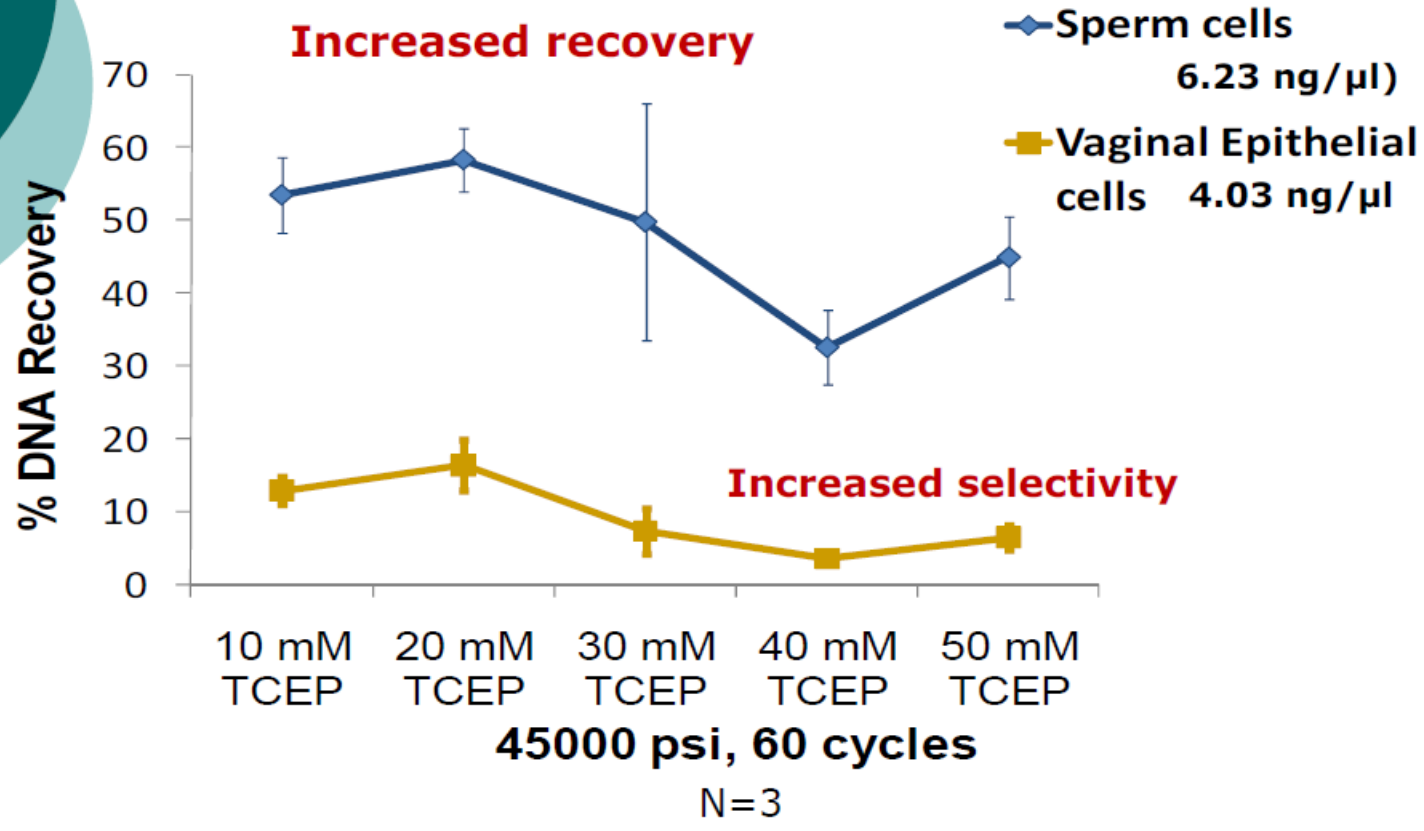
TCEP studies



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Next step:
Mixtures and dried stains

- Key issues
- Switch to Plexor HY to permit simultaneous quantification of both autosomal and Y DNA
- Examination of liquid and dried samples.
- Key issues: Developing accurate quantification, reoptimization of method for dried stains.

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Conclusions

- PCT treatment can produce selective extraction of epi and sperm cells
- Depending on buffer component epi or sperm cells can be selectively lysed
- TCEP produces improved selectivity of sperm extraction
- Combining TCEP and DTT further improves yield in case of mixed samples
- Increasing pressure cycles above 60 has no effect on yield

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CSI-NY

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Thank you!

Pressure Biosciences Inc.

14 Norfolk Avenue
South Easton, MA 02375
Midwest Office Phone: (608) 395-7106
email: tstrader@pressurebiosciences.com