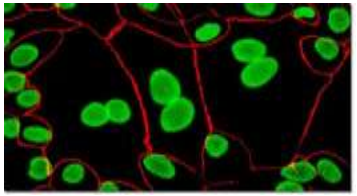
FIU

International Forensic Research Institute

Application of Pressure Cycling Technology (PCT) in Differential Extraction



Deepthi Nori, MFS; Dr. Bruce R. McCord, PhD

Department of Chemistry

International Forensic Research Institute

Florida International University

Overview

- Pressure Cycling Technology (PCT)
- Differential Extraction
- Objective
- Experimental design
- Results
- Conclusions

Pressure Cycling Technology

- Barocycler® NEP2320 (Pressure BioSciences Inc.)
- User controlled variables:

a) Pressure (5-45k psi)

b) Holding time at ambient pressure (T1)

c) Holding time at target pressure (T2)

d) No. of cycles (1-99)

e) Temperature

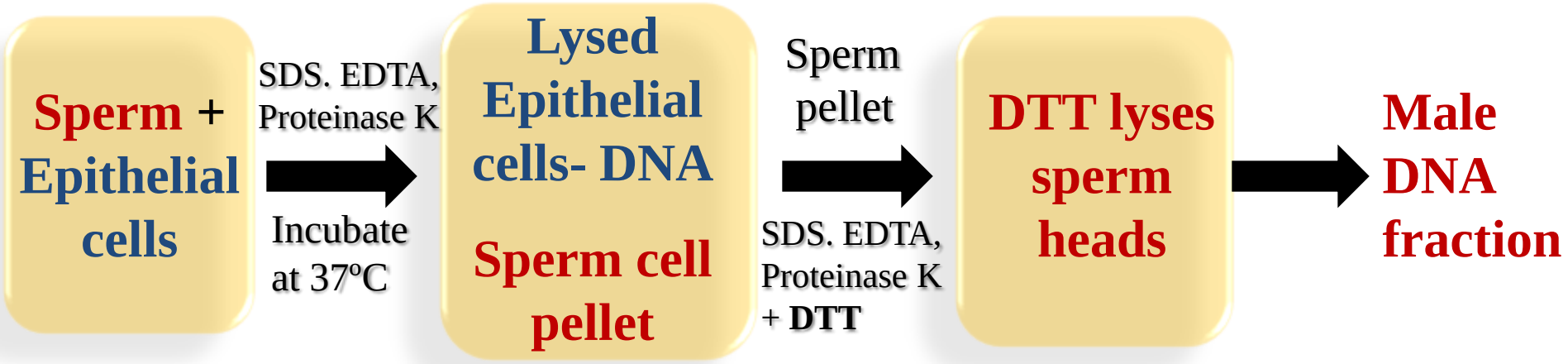
PULSE™ tubes (up to 300 µl)

MicroTubes (50-150 µl)

Pressure BioSciences Inc.

Differential Extraction

- Sexual assault casework
- Separation of female-derived sperm cells and epithelial cells



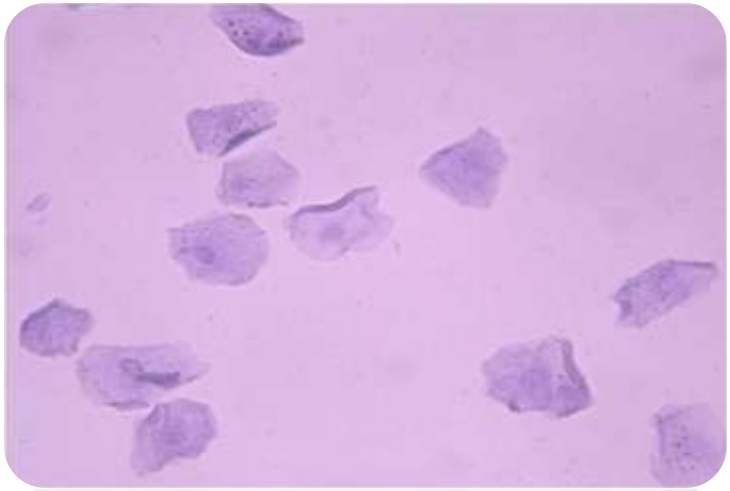
- Male DNA profile

Objective

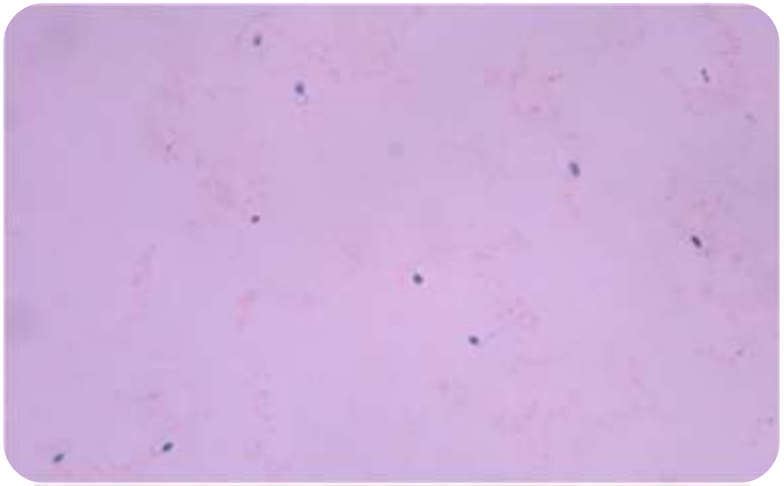
- **Conventional Differential Extraction**
  - Digestion of Epithelial cells
  - Isolation of Sperm cells
- **Pressure-based extraction**
  - Intact Epithelial cells
  - Selective lysis of Sperm cells

PCT- Microscopic studies

- Microscopic visualization of PCT-treated cells stained with 0.4% Trypan blue solution
- Trypan blue staining - dye exclusion method
- Cells with a compromised membrane integrity are stained dark blue



Vaginal epithelial cells



Sperm cells

Experimental design

SAMPLES

Sperm cells and Epithelial cells suspended in 1X PBS buffer (pH 7.4)

CELL LYSIS

Pressure cycling using Barocycler® NEP 2320

PURIFICATION

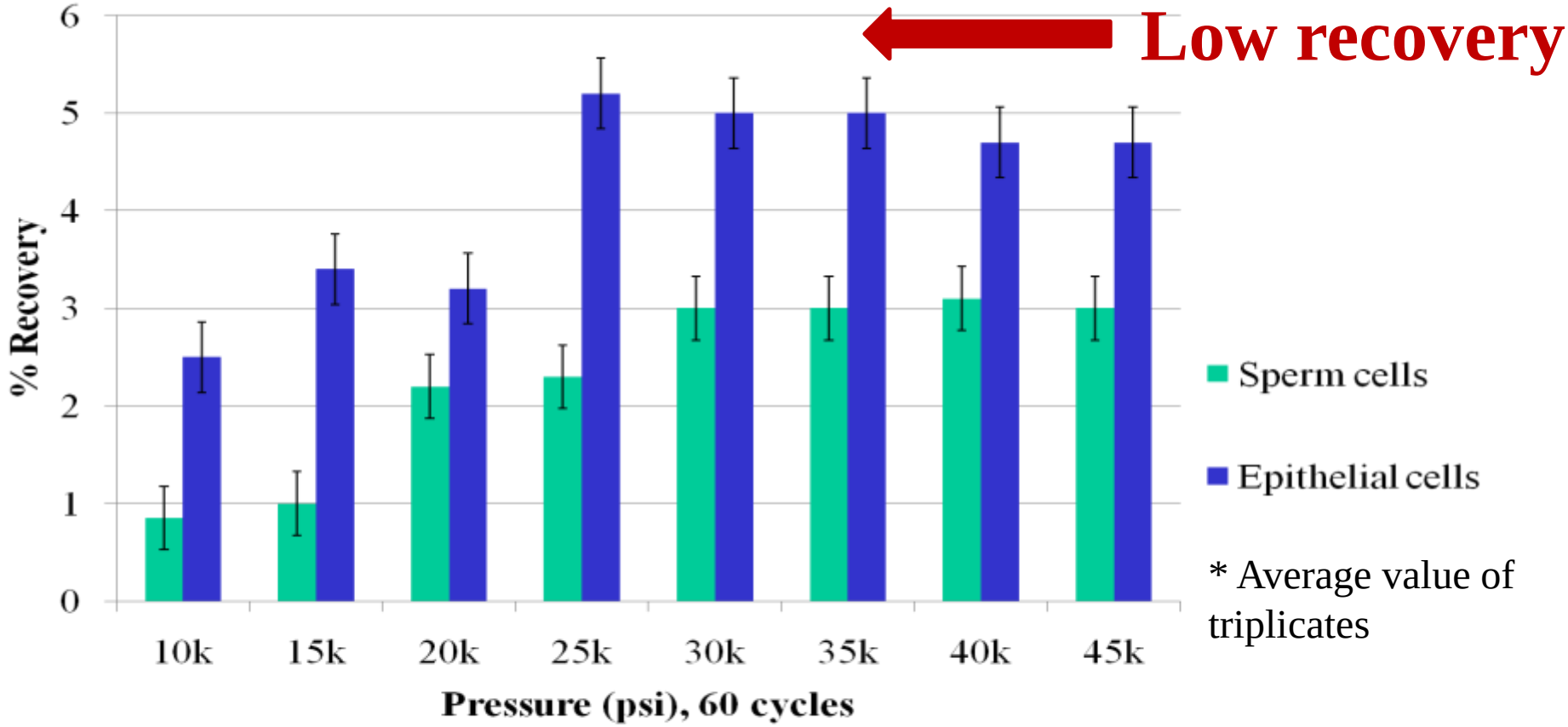
Phenol-Chloroform-Isoamyl alcohol extraction

QUANTITATION

Real-time PCR analysis using Plexor® HY system (Promega Corporation)

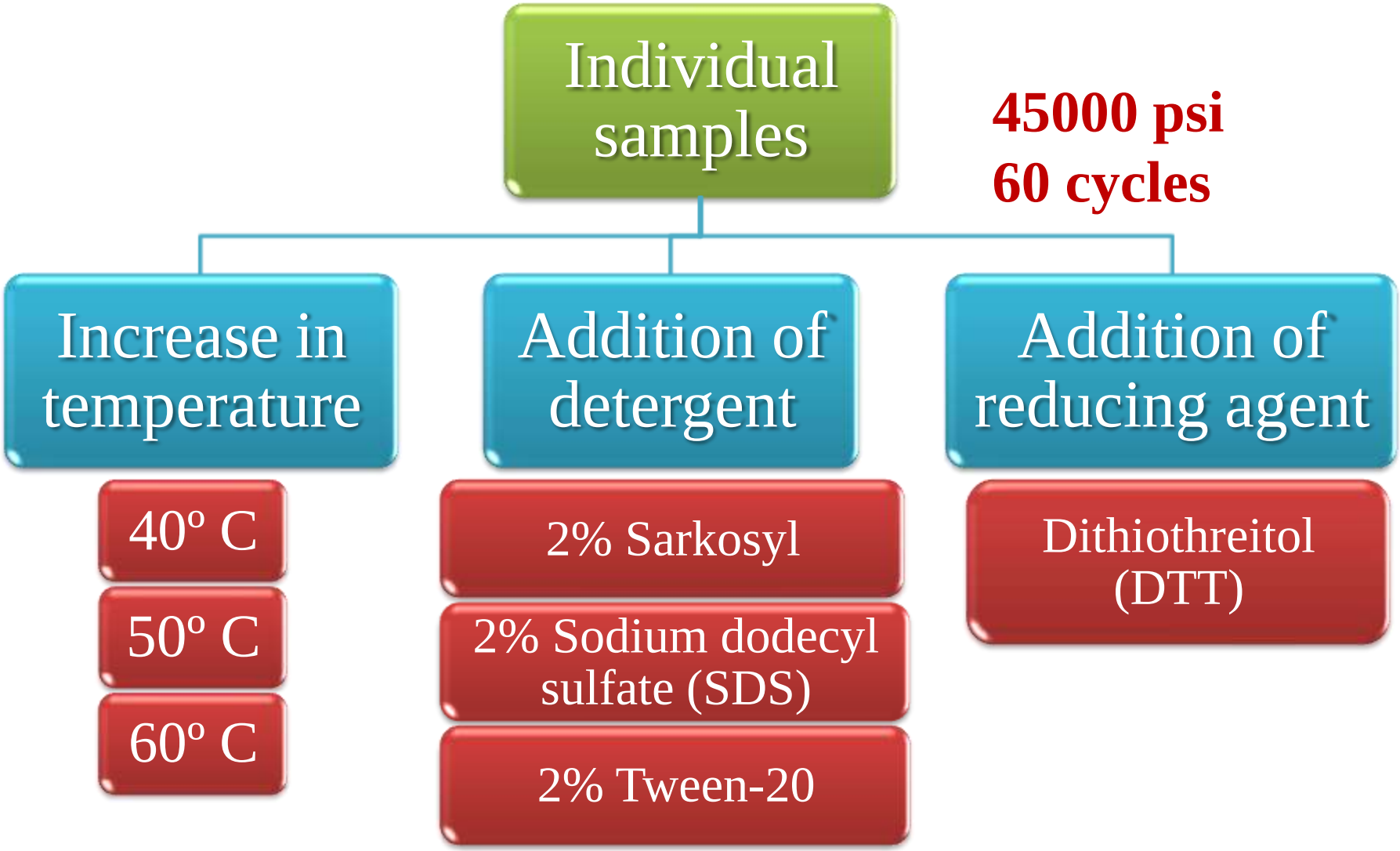
Dose Response curve

Total DNA in sample- Standard Phenol/Chloroform and Proteinase K extraction

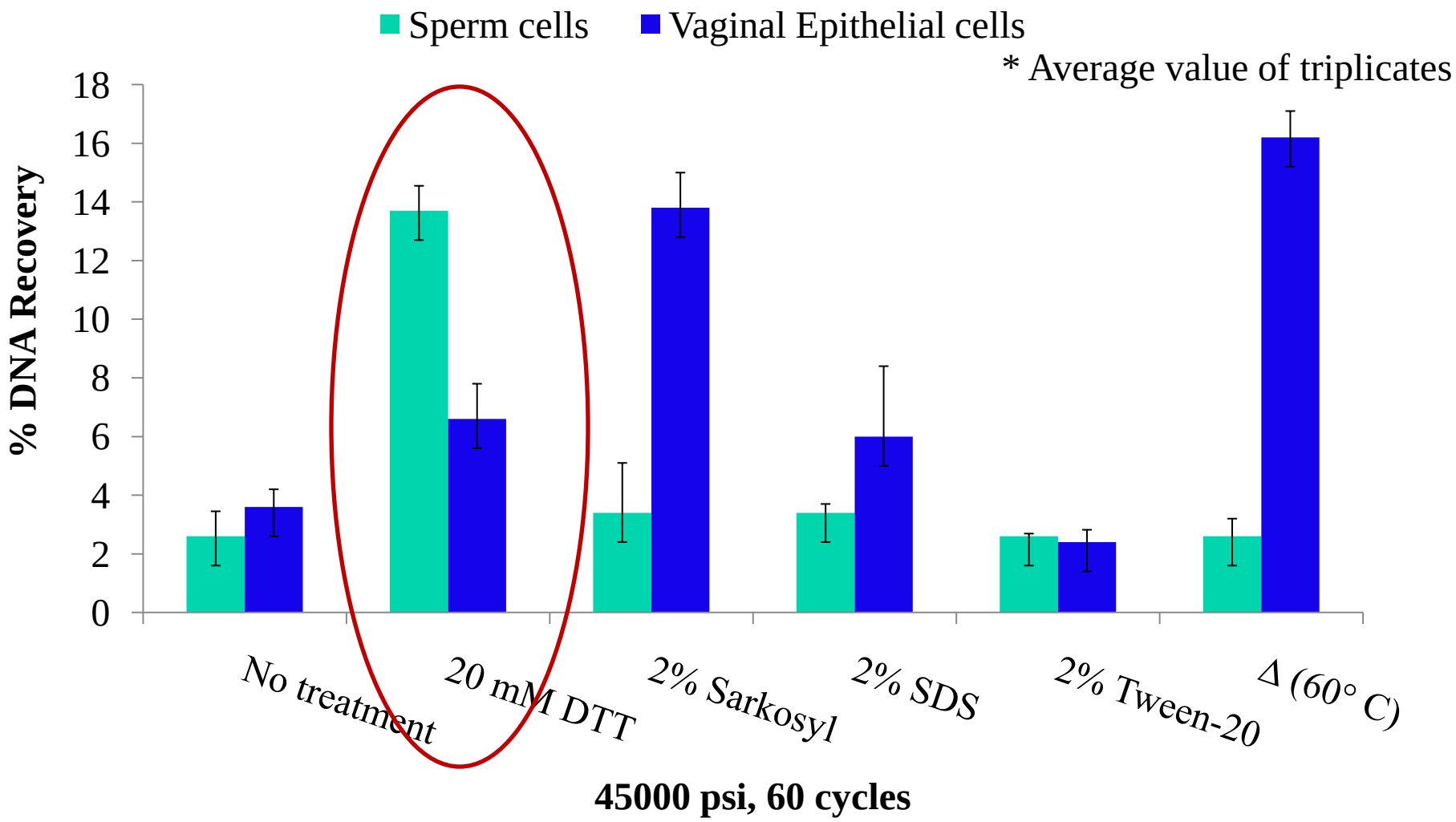


Results presented at 21st International Symposium on Human Identification

Maximize DNA recovery



Effect of PCT on treated cells



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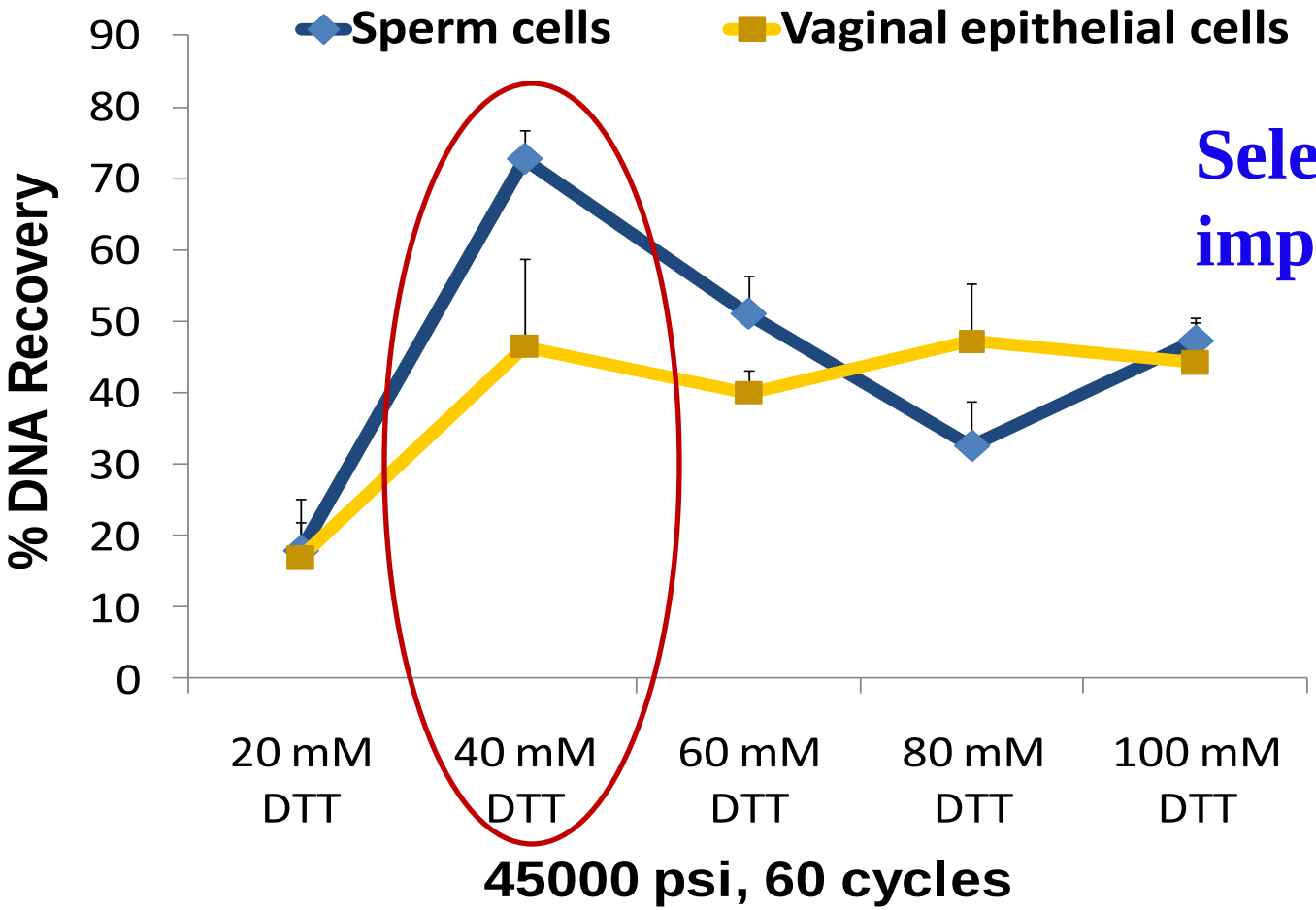
Dithiothreitol (DTT) studies

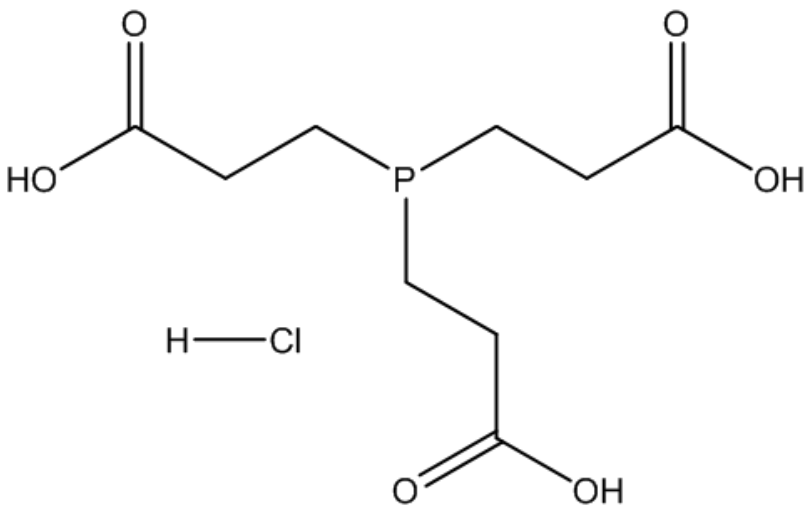


Selectivity needs improvement

* Average value of triplicates

Tris(2-carboxyethyl)phosphine (TCEP)

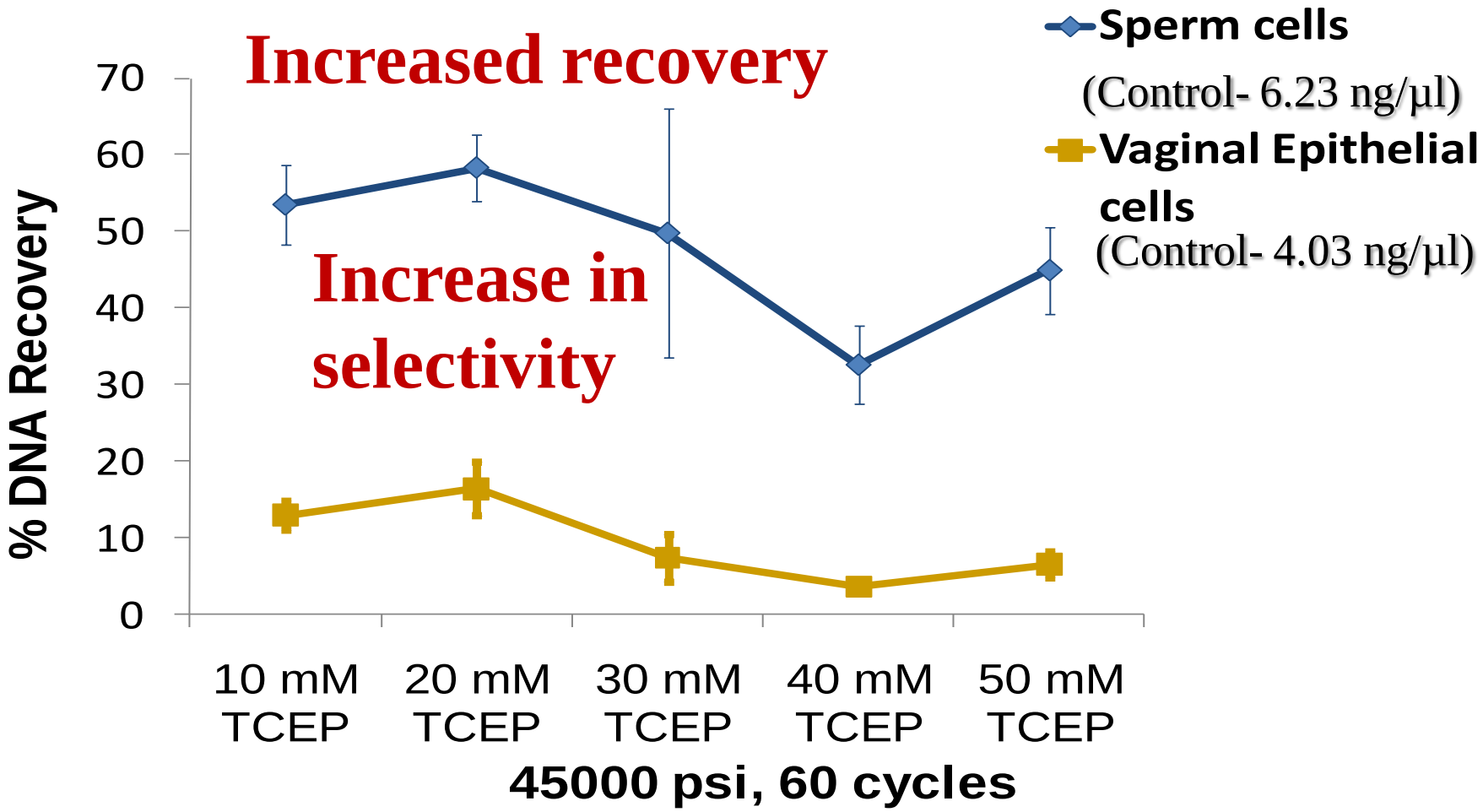
- Soluble in water
- More effective than DTT
- Odorless
- Selectively reduces disulfide bonds over a broad pH range
- Stable- resistant to air oxidation
- TCEP is not particularly stable in phosphate buffers



Chemical Formula:	$C_9H_{16}O_6PCl$
Molecular Weight:	286.6 g/mol

Thermo Fisher Scientific Inc.

TCEP studies



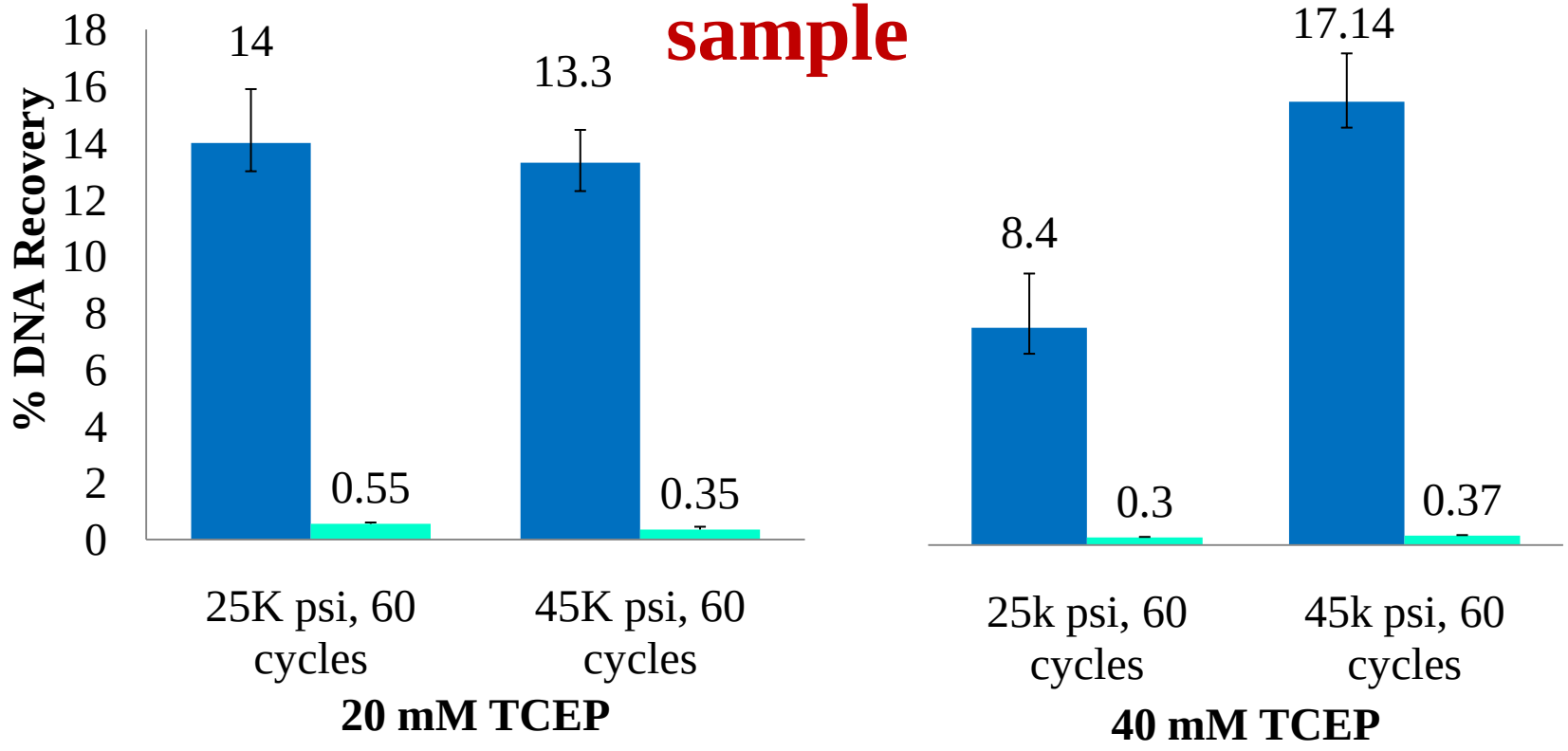
* Average value of triplicates

TCEP studies

Sperm cells (Control- 32.7 ng/µl)

Vaginal Epithelial cells (Control- 40.3 ng/µl)

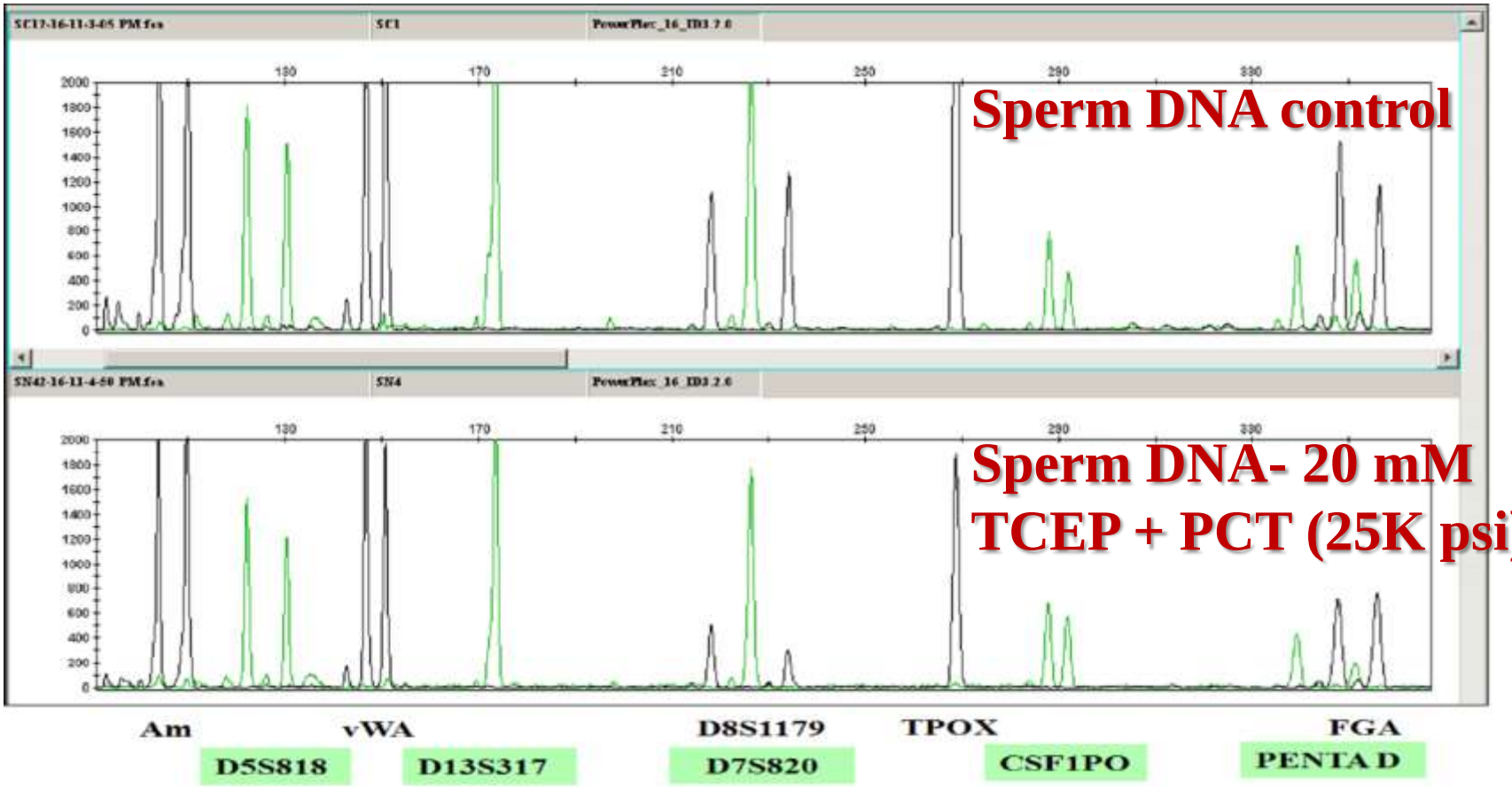
Selectivity reproducible at higher concentrations of sample



* Average value of triplicates

STR profile

PowerPlex® 16 HS (Promega Corporation)



Conclusions

- Pressure Cycling Technology (PCT) - Potential application in differential extractions
- Tris (2-carboxyethyl) phosphine (TCEP)
  - High selectivity
  - Improved recovery
  - Selective detection of sperm cells
- Successful amplification of sperm DNA recovered from pressure lysis
- Future work- Mixtures and dried stains

Acknowledgements

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Vera Gross, PhD: Senior Scientist

Promega Corporation

Len Goren, Promega Genetic Identity Director

Broward Sheriff's Office DNA Laboratory

Dr. George Duncan