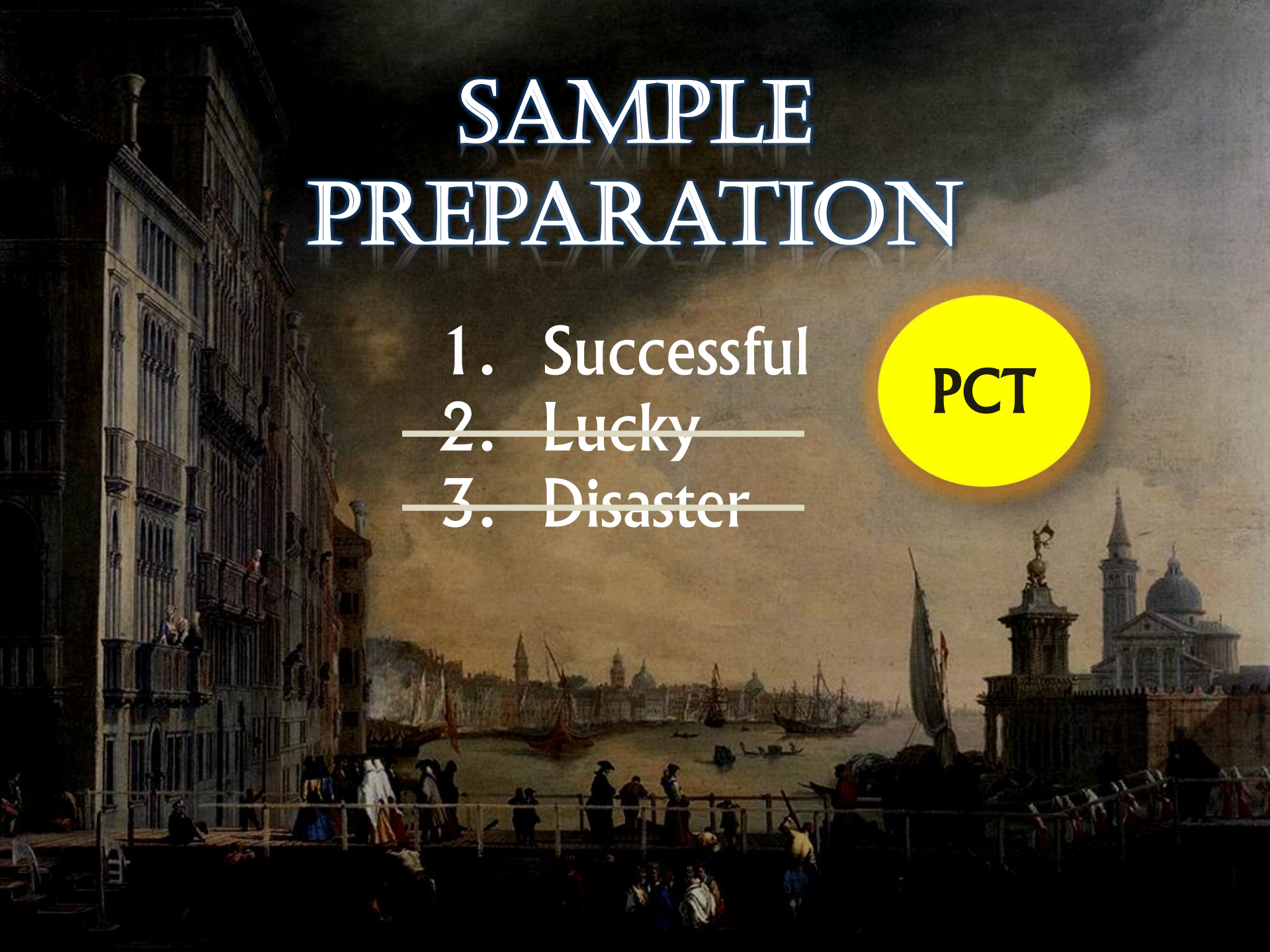
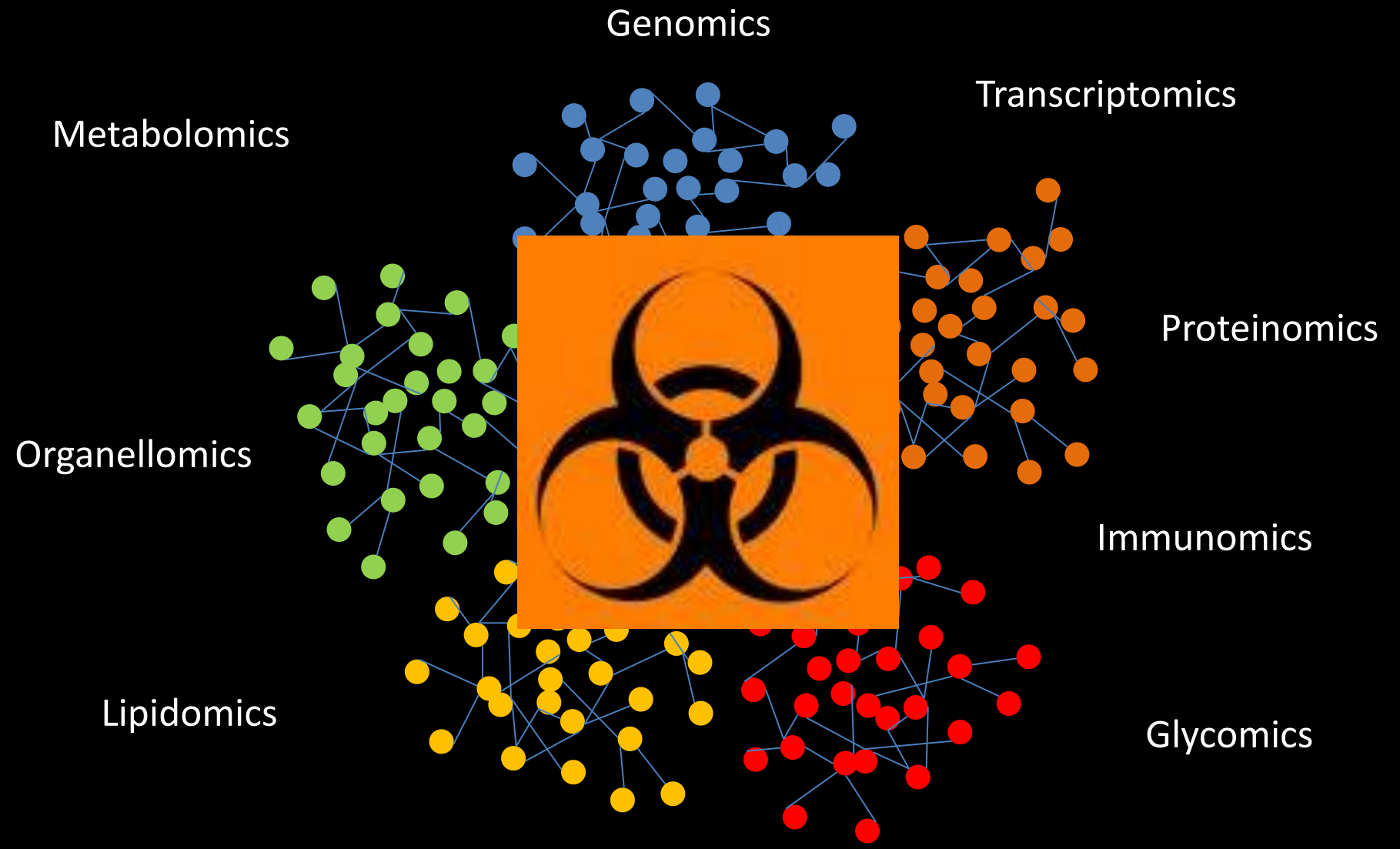
Optimal Preparation of Difficult Samples for Systems Biology

Bradford Powell Ph.D., PMP

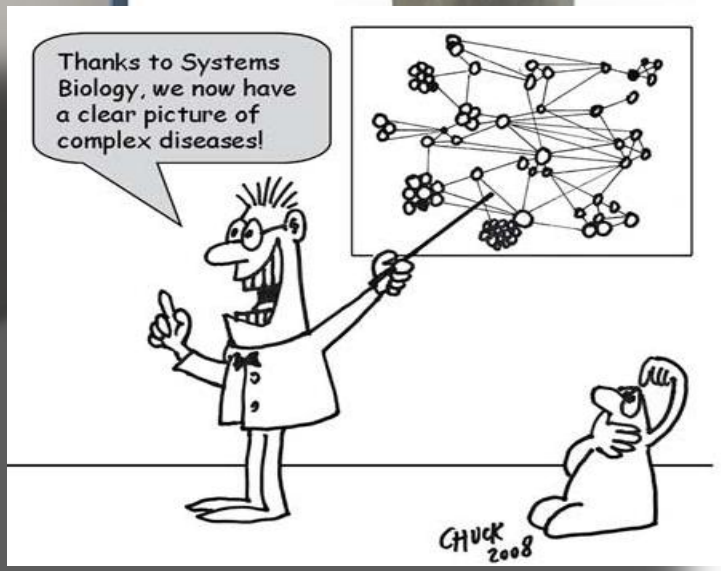
Cernomics Solutions

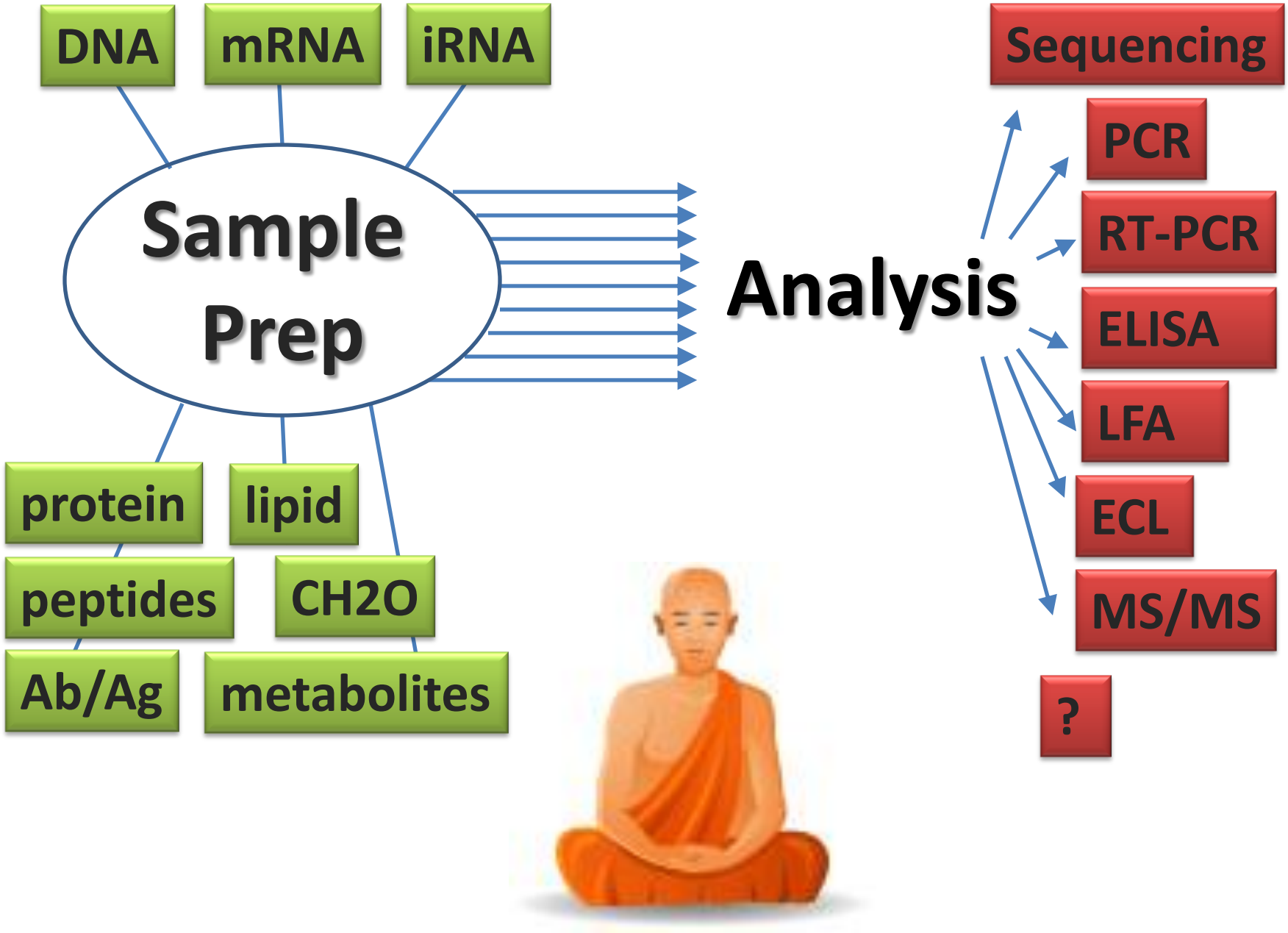

SAMPLE PREPARATION
1. Successful
2. Lucky
3. Disaster
PCT

Infectious Samples for Systems Biology?



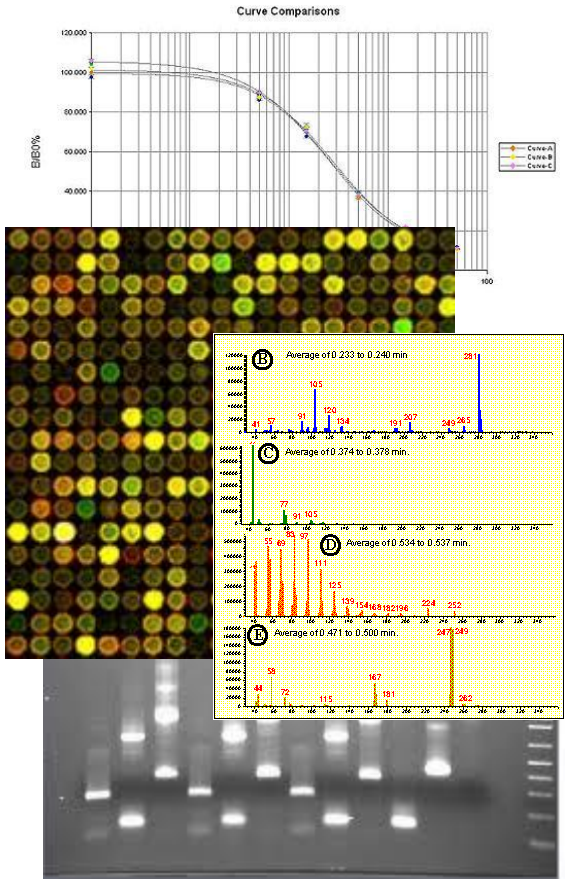
Sample Preparation




DNA
mRNA
iRNA
Sample Prep
protein
lipid
peptides
CH2O
Ab/Ag
metabolites
Analysis
Sequencing
PCR
RT-PCR
ELISA
LFA
ECL
MS/MS
?

BSL-3/-4

BSL-2


Curve Comparisons
Curve-A
Curve-B
Curve-C
Average of 0.233 to 0.240 min.
Average of 0.374 to 0.378 min.
Average of 0.534 to 0.537 min.
Average of 0.471 to 0.500 min.

Biothreat Microbe Inactivation

Inactivation Method	Molecular Analysis	Comments
Autoclave	Partial	PCR; Physical hazard, facilities, instrument & training requirements
γ-irradiation	Partial	Antigen detection; Physical hazard, facilities, instrument & training requirements
Formaldehyde	No	Antigen detection; Chemical hazard, Facility & training requirements
UV-irradiation	No	Poor inactivation; instrument & training requirements
Ethylene oxide	No	Chemical hazard, facilities, instrument & training requirements
*TriZol™/$CHCl_3$	Partial	Nucleic acids; chemical hazard
Bead Beater	Partial	Unsafe, known breaches
Filtration	Yes	Unsafe, documented incidences
PCT	**Yes**	**Safe, Under development**

*TriZol extraction contains guanidinium thiocyanate, phenol and chloroform. Positive strand virus (e.g., VEE) requires additional care and treatment due to infectivity of RNA.

PCT safely* inactivates BSAT samples

Approach	Inactivation		Analysis Modes
	Spores [1]	Virus[2]	
PCT	8 log	$4.4x10^6$	PCR, RT-PCR, Sequencing, LC-MS/MS, WB
FMT	4 log	nt	nt

* Preserve personnel safety and sample molecular integrity

[1] *Bacillus anthracis* Sterne spores, cfu/ml decrease

[2] Venezuelan equine encephalitis virus (VEE) TC83, PFU/ml limit

Related Publications

1. Bradford S. Powell and Loreen L. Lofts. Need for improved sample inactivation and extraction methods to support systems biology analysis of biological threat organisms. Abstracts Applications of ultra-high pressure in biotechnology. Expert Reviews Prot. 2010 7(suppl. 4): 18
2. Bradford Powell and Robert Cybulski. “Inactivation and extraction of bacterial spores for systems biological analysis” p. 941-977. In, A. Ivanov, A. Lazarev (ed), **Sample Preparation in Biological Mass Spectrometry**, Springer, New York, 2011.
3. Powell BS, Lazarev AV, Carlson G, Ivanov AR, Rozak DA. “Pressure Cycling Technology in Systems Biology” in **Microbial Systems Biology: Methods and Protocols**, ed. Navid A, New York: Humana Press 2012.

Pressure Cycling Technology

PCT is a novel, **enabling** technology that uses cycles of hydrostatic pressure between atmospheric and Ultra-high Levels to **Safely Manipulate** the Physico-Chemical Control of **Biomolecular Interactions**.

- **Microbe Inactivation**
- **Molecule Extraction**
- **Differential Lysis/Partition**
- **Enzyme Kinetics/Kill**

Pressure BioSciences Inc.

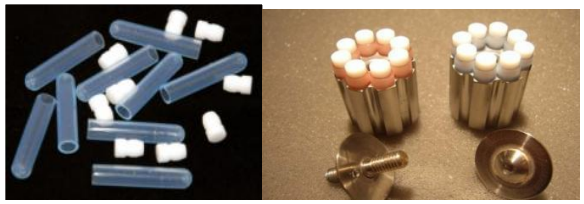
Barocycler™ NEP3229

Barocycler™ NEP2320

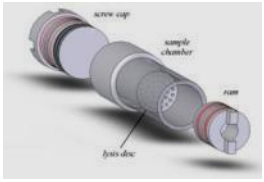
Barocycler™ HUB440

PCT Shredder and The Shredder SG3

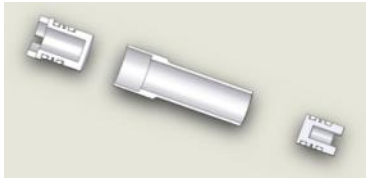
PCT MicroTubes



PULSE Tube FT500



PULSE Tube FT500-ND

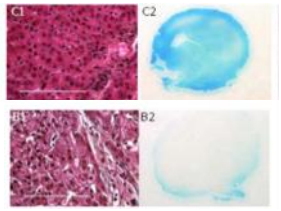


Reagent Kits

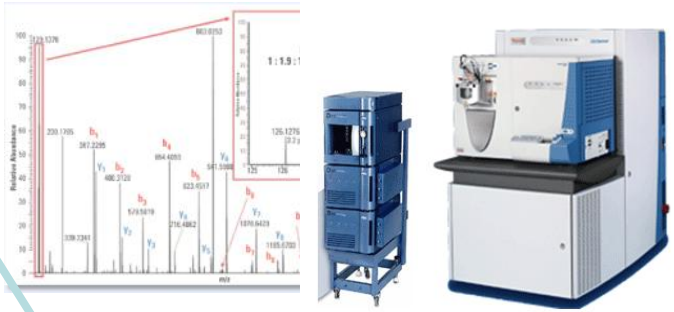
PCT Applications

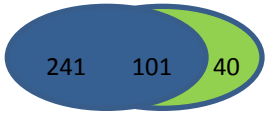
FORENSIC DNA EXTRACTION

PATHOLOGY SAMPLE PREPARATION

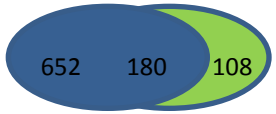


ACCELERATED ENZYMATIC DIGESTION





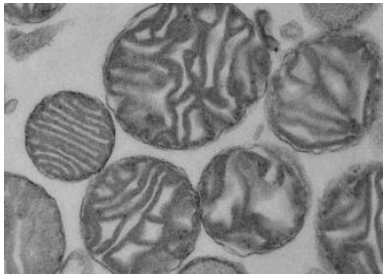
proteins



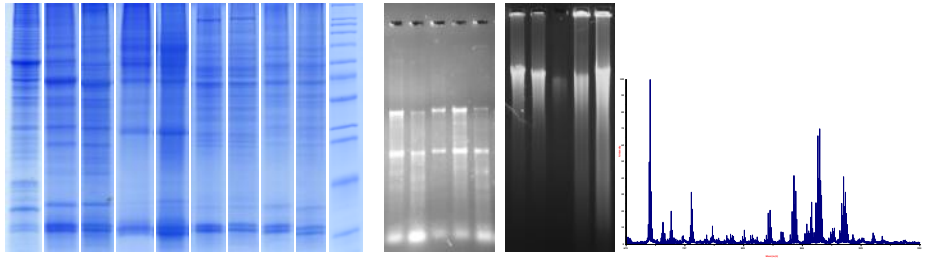
peptides

CELL AND TISSUE LYSIS

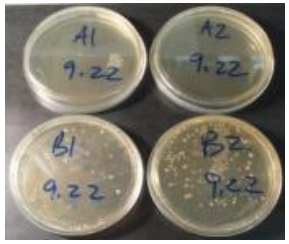
SUBCELLULAR FRACTIONATION



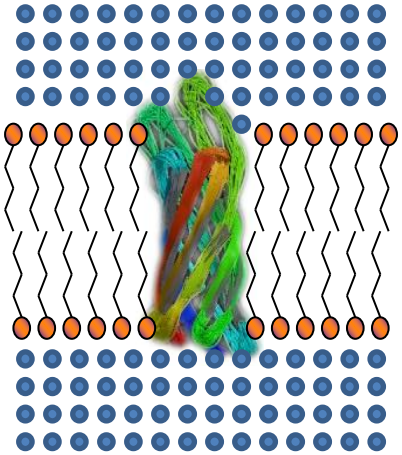
EXTRACTION AND FRACTIONATION DNA, RNA, LIPIDS AND PROTEINS



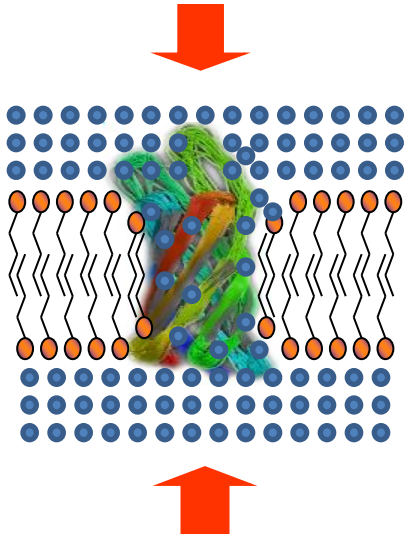
PATHOGEN INACTIVATION



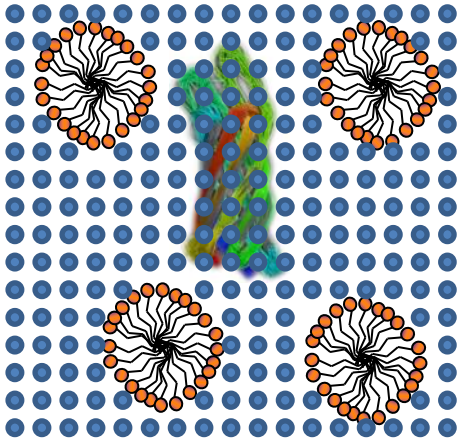
PCT Destabilizes Biological Membranes



Membrane Protein in Lipid bilayer



Hydrostatic Pressure Applied



Hydrostatic Pressure Released

1. Interdigitated bilayer
2. Hydrophobic hydration

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

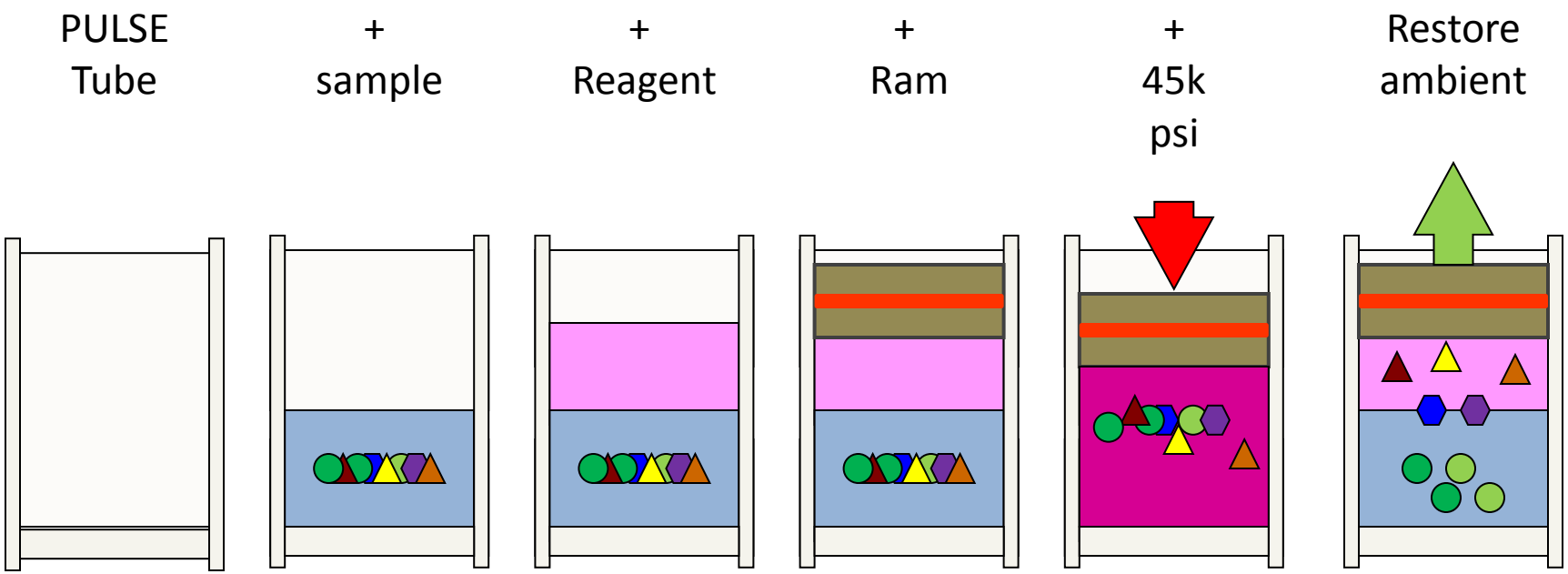
Marianas Trench:
38,713 ft (11,800m) deep
16,000 PSI (120 MPa)

Medically Important Infectious Microbes and Mammalian Hosts Evolved at STP

Barocycler & PULSE tubes
45,000 PSI (310 MPa)

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

PCT-mediated liquid-liquid extraction



Gross, *et al*., JBT (2008) 19*:187–197*

PCT-ProteoSolve Workflow

PULSE Tube + SAMPLE + REAGENTS

ΔT ΔP controlled

PCT-assisted chemistry

PCT-assisted NA amplification

PCT-assisted digestion

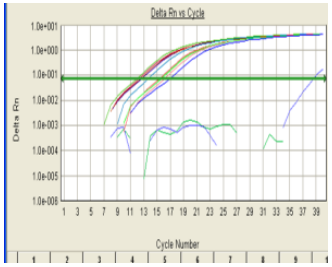
CENTRIFUGATION ~12,000g for 10 min.

Pellet: Nucleic Acids

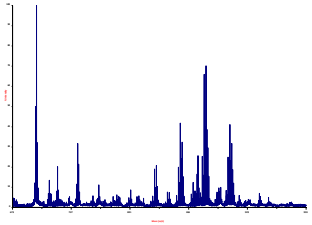
Top Phase: Lipids

Bottom Phase: Proteins

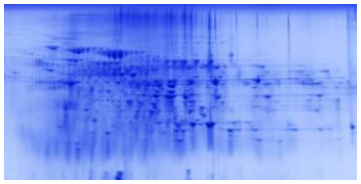
GENOMICS:
DNA Sequencing
SNPs, RNAi



LIPIDOMICS:
GC-MS, LC-MS,
MALDI/TOF
TLC, NMR

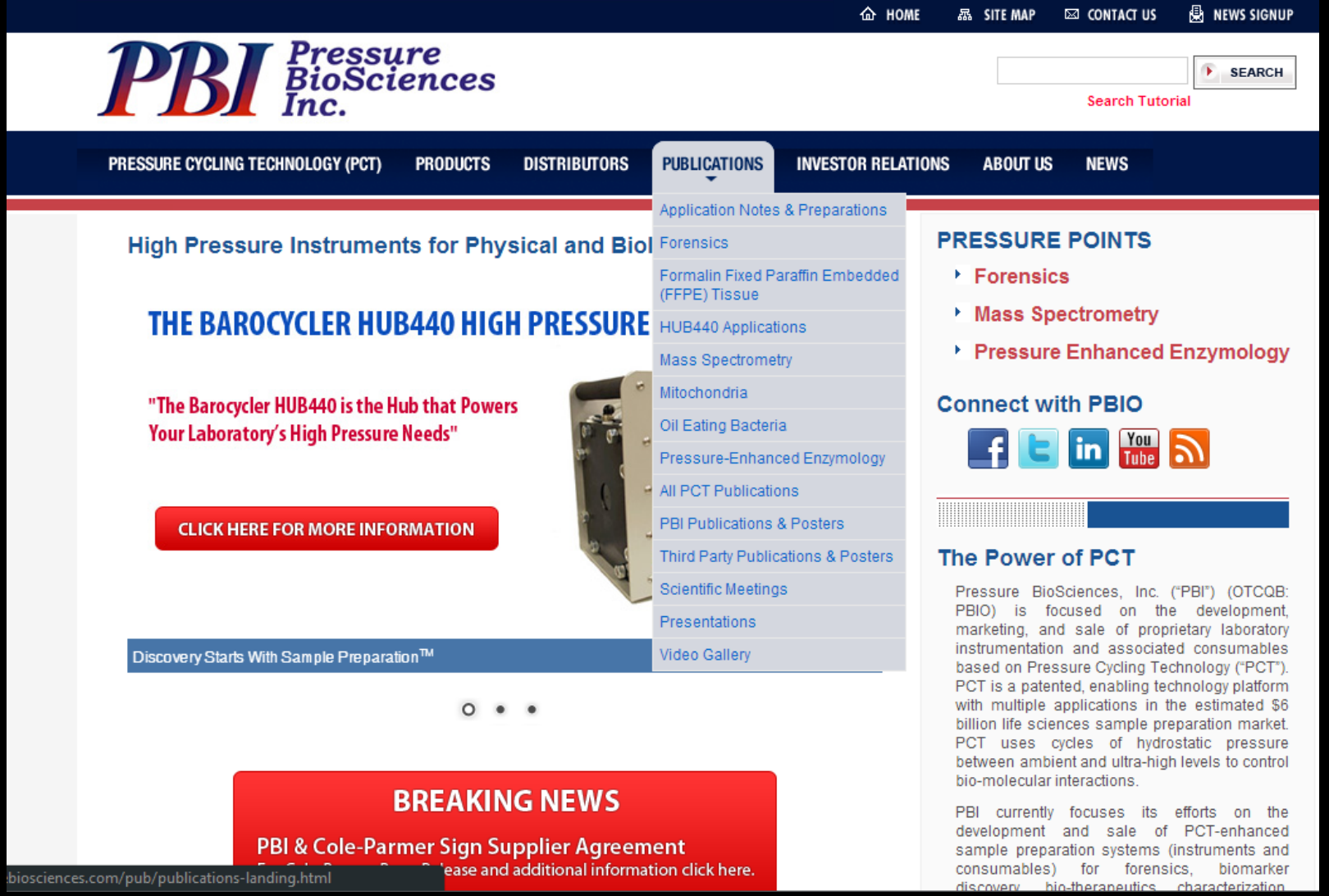


PROTEOMICS
SDS-PAGE, 2D PAGE,
Western blotting,
LC-MS/MS,
MALDI-TOF, etc...



PCT

- All sample types, including biothreats
- All molecular analytes (NA, protein, lipid, …)
- Leverage limited sample for multiplex
- Simplified reporting, transport & archival
- Accelerated work-up (enzymology)



www.pressurebiosciences.com

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>200 Published Applications of PCT

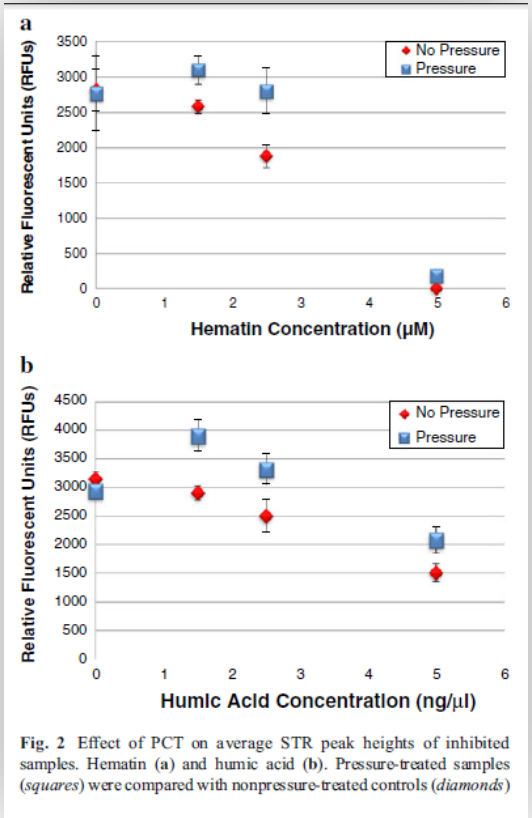

Fig. 2 Effect of PCT on average STR peak heights of inhibited samples. Hematin (**a**) and humic acid (**b**). Pressure-treated samples (*squares*) were compared with nonpressure-treated controls (*diamonds*)

Int J Legal Med
DOI 10.1007/s00414-012-0770-y

ORIGINAL ARTICLE

Pressure cycling technology (PCT) reduces effects of inhibitors of the PCR

Pamela L. Marshall · Jonathan L. King · Nathan P. Lawrence · Alexander Lazarev · Vera S. Gross · Bruce Budowle

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Abstract A common problem in the analysis of forensic human DNA evidence, or for that matter any nucleic acid analysis, is the presence of contaminants or inhibitors. Contaminants may copurify with the DNA, inhibiting downstream PCR or they may present samples effectively as containing fewer templates than exist in the PCR, even when the actual amount of DNA is adequate. Typically, these challenged samples exhibit allele imbalance, allele dropout, and sequence-specific inhibition, leading to interpretational difficulties. Lessening the effects of inhibitors may increase the effective yield of challenged low template copy samples. High pressure may alter some inhibitors and render them less effective at reducing the yield of PCR products. In an attempt to enhance the amplicon yield of inhibited DNA samples, pressure cycling technology was applied to DNA exposed to various concentrations of hematin (0, 1.25, 2.5, 5, and 7 μM) and humic acid (0, 1.25, 2.5, 5, and 7 ng/μL). The effect of high pressure on the inhibitors and subsequently the PCR process was assessed this method can improve the ability to type challenged or inhibited DNA samples.

Keywords PCR inhibition · Pressure cycling technology · Forensic DNA analysis · Hematin · Humic acid · Hi-Flow column

Introduction

Samples that contain a suboptimal quantity and/or limited quality of DNA are commonly encountered in forensic DNA analyses. Several materials act as potent inhibitors of PCR, including collagen, calcium ions, melanin, hematin, and humic acids found in soil [1, 2]. Many inhibitory substances may copurify with the DNA and impact the PCR process. Inhibition has been shown to affect the accuracy of template quantification by quantitative PCR (qPCR) [3]. In

Improved extraction of *Rhizoctonia* and *Pythium* DNA from wheat roots and soil samples using pressure cycling technology

Richard T. Schumacher,

the dryland cereal production region of
n spp. are causal agents of root rot,
vulgare). Although these pathogens can
ction of *Rhizoctonia* DNA from
densities. Using a novel extraction
of *R. solani* AG-8 DNA up to 16-fold

Table 3. Comparison of pressure cycling technology (PCT) and bead beating (BB) extraction of *Rhizoctonia solani* AG-8 and *Pythium abappressorium* DNA from agricultural soils from Ritzville, Washington.

		R. solani AG-8		*P. abappressorium*	
Sample[a]	Treatment[b]	DNA (pg/g soil)[c]	PCT/BB ratio	DNA (pg/g soil)	PCT/BB ratio
T4 R3a	PCT	551±94	3.6	2.3±0.24	1.0
	BB	152±68		2.3±0.07	
T5 R2d(c)	PCT	258±36	16	33±10	1.8
	BB	16±10		18±6.7	
T9-06 R3b	PCT	515±4.8	7.3	9.6±0.98	2.0
	BB	71±14		4.8±0.73	
T9-06 R3c	PCT	432±45	5.6	35±1.2	1.5
	BB	76±13		23±3.4	

Note: Soil type was classified as Ritzville silt loam (USDA Natural Resources Conservation Services website).

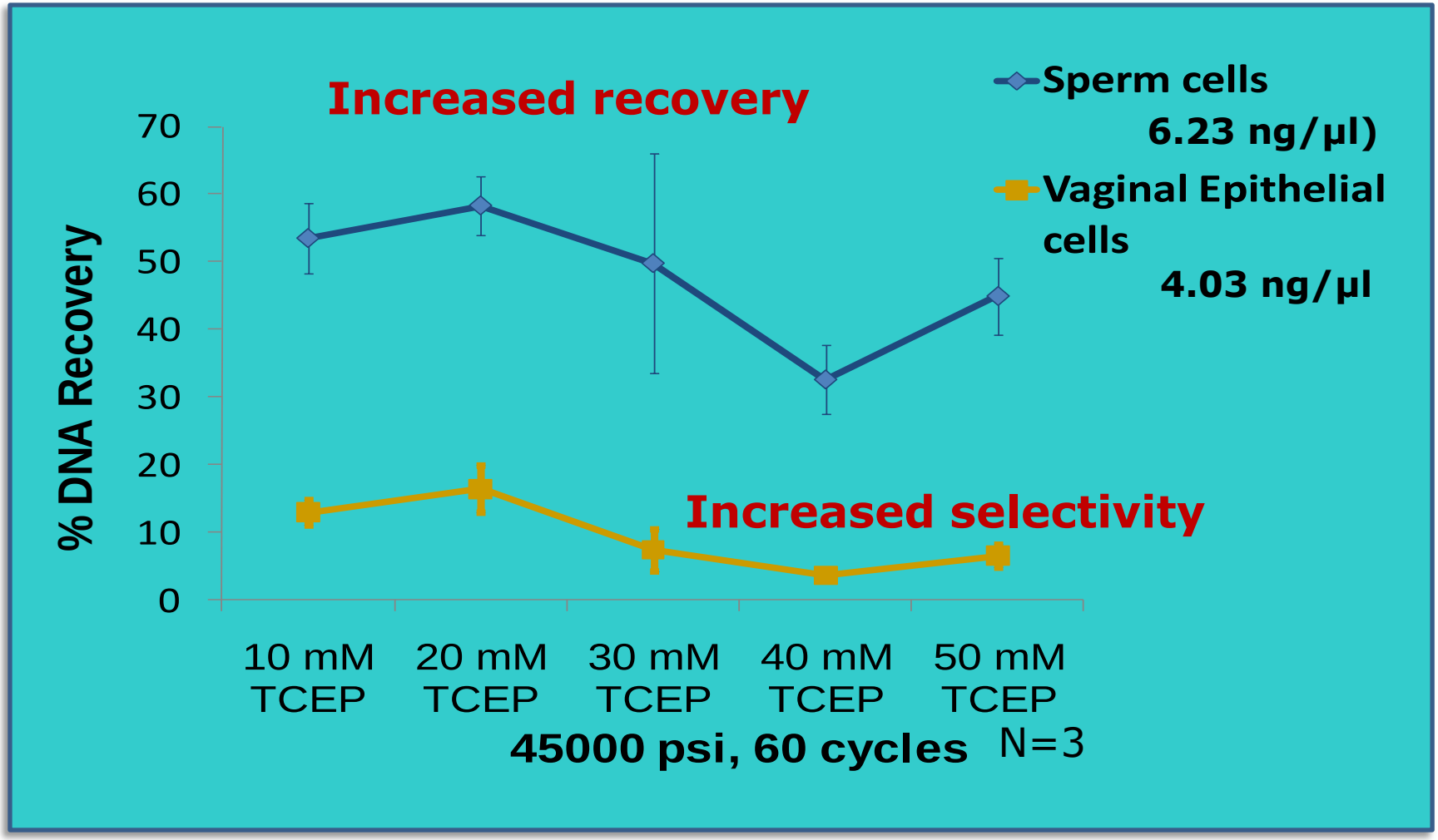
[a]Soil samples designated T* were collected from Ritzville, Washington, in September 2006.

[b]Pressure cycling was performed using 15 cycles of 35 000 psi (235 MPa) for 20 s followed by ambient pressure for 10 s for 15 cycles. Bead beating extraction was done using the UltraClean soil DNA kit and FastPrep FP120 homogenizer.

[c]Means and SEs of triplicate extractions of 0.2 g soil (PCT) or 0.5 g soil (BB), determined from standard curves of fungal genomic DNA added to soil extracts. Standard curve equations were $C_t = -3.805 \log(\text{pg DNA}) + 28.388$ for *R. solani* AG-8 and $C_t = -3.749 \log(\text{pg DNA}) + 24.987$ for *P. abappressorium*, where C_t is the mean cycle threshold value of two Q-PCR reactions.

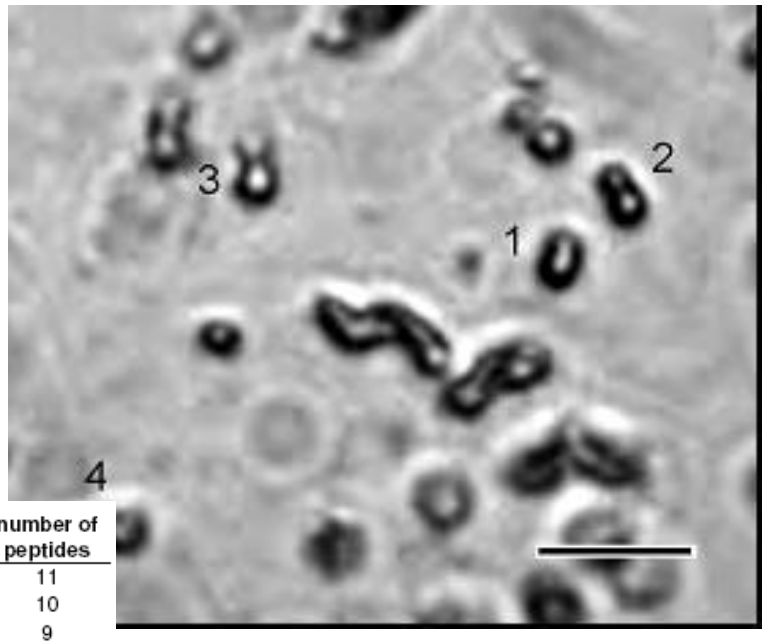
Table 4. Comparison of pressure cycling technology (PCT) and bead beating (BB) extraction of *Rhizoctonia solani* AG-8 DNA from additional agricultural soils.

Closing Sexual Assault Sample Analysis Backlog

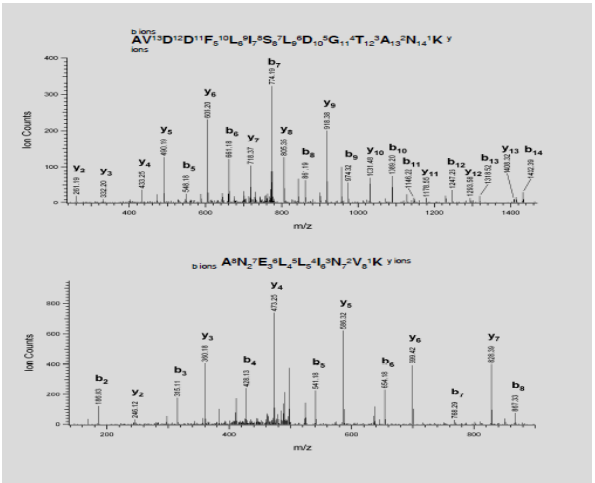


Deepthi Nori and Bruce R. McCord, Department of Chemistry and Biochemistry, Florida International University mccordb@fiu.edu www.fiu.edu/~mccordb

Proteomics from Oligo-Meiocene amber sample



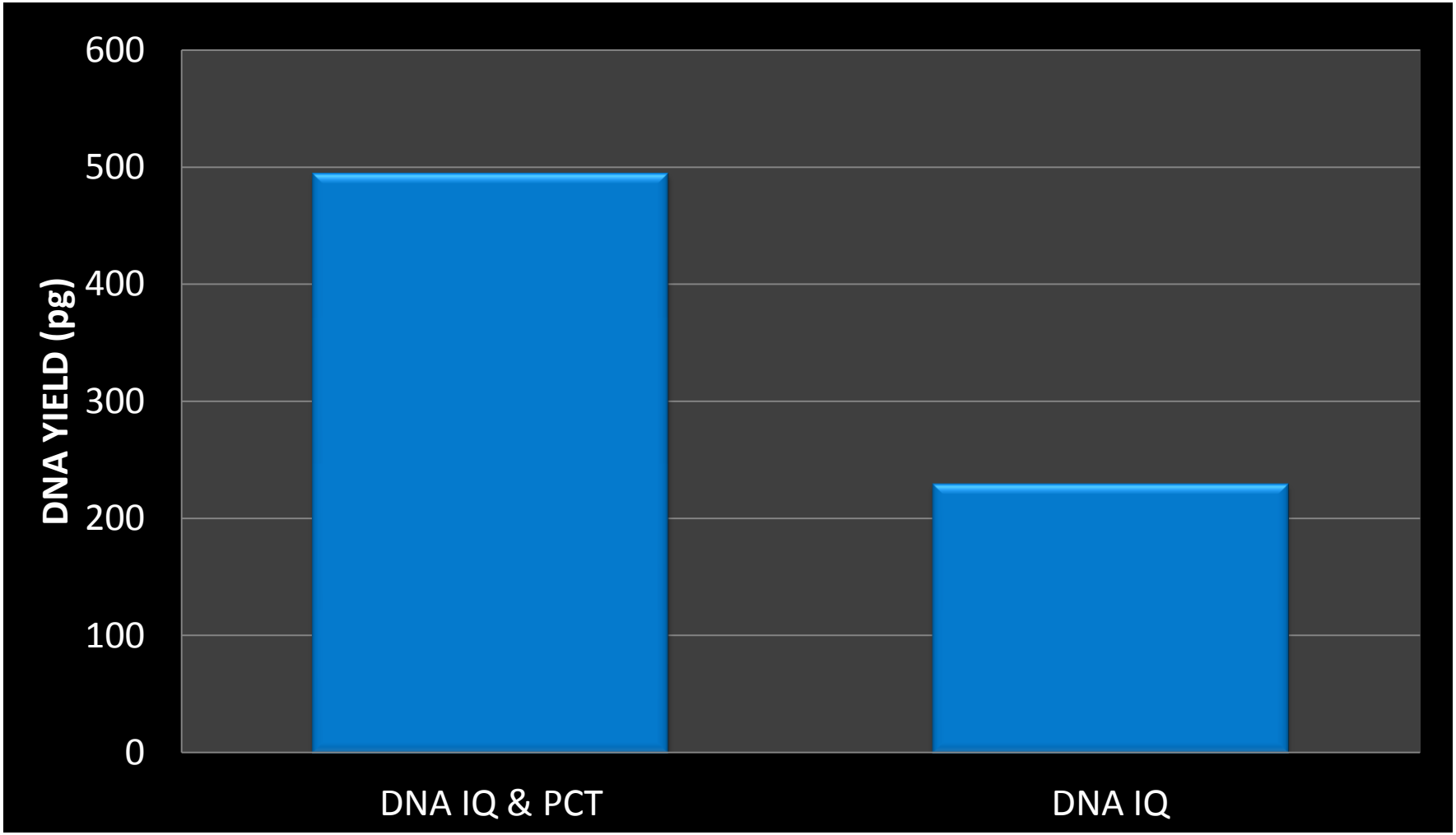
	protein identification	number of peptides
1	enolase 1	11
2	enolase 2	10
3	alcohol dehydrogenase	9
4	glyceraldehyde-3-phosphate dehydrogenase, isozyme 1	8
5	phosphoglycerate kinase	7
6	translation elongation factor EF-1 alpha	7
7	pyruvate kinase	5
8	glyceraldehyde-3-phosphate dehydrogenase, isozyme 3	3
9	heat shock protein YJM789	3
10	fructose 1,6-bisphosphate aldolase	3
11	phosphoglycerate mutase	3
12	ribosomal L12B	2
13	triosephosphate isomerase	2
14	methionine and cysteine synthase (O-acetyl homoserine-O-acetyl serine sulfhydrylase)	2
15	Ssa1p	2
16	mitochondrial aldehyde dehydrogenase	2
17	rubber elongation factor protein	2
18	actin	2
19	40S ribosomal protein S18	2
20	pyruvate decarboxylase	1
	total	86



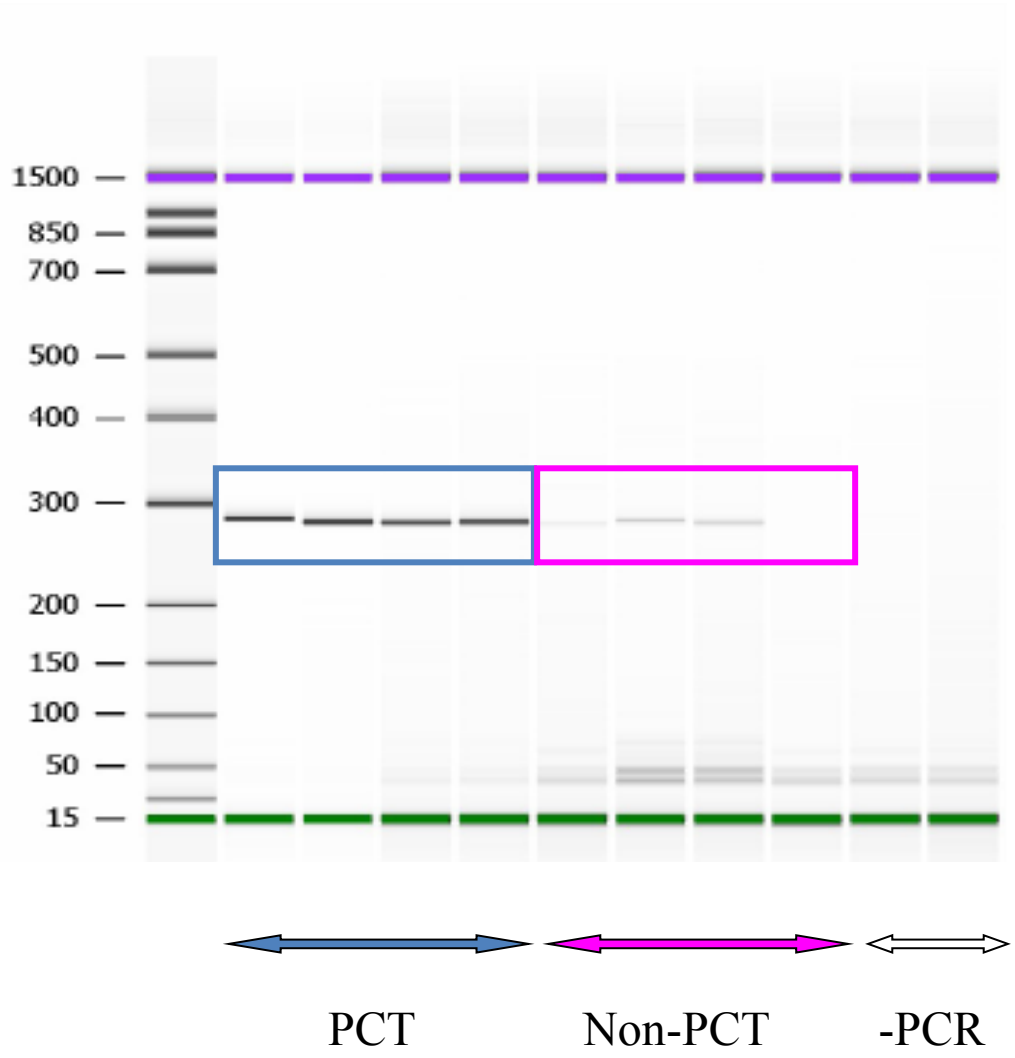
G. Smejkal, Pressure Symposium, Harvard University, May 21, 2010

Forensics from scarce & difficult samples

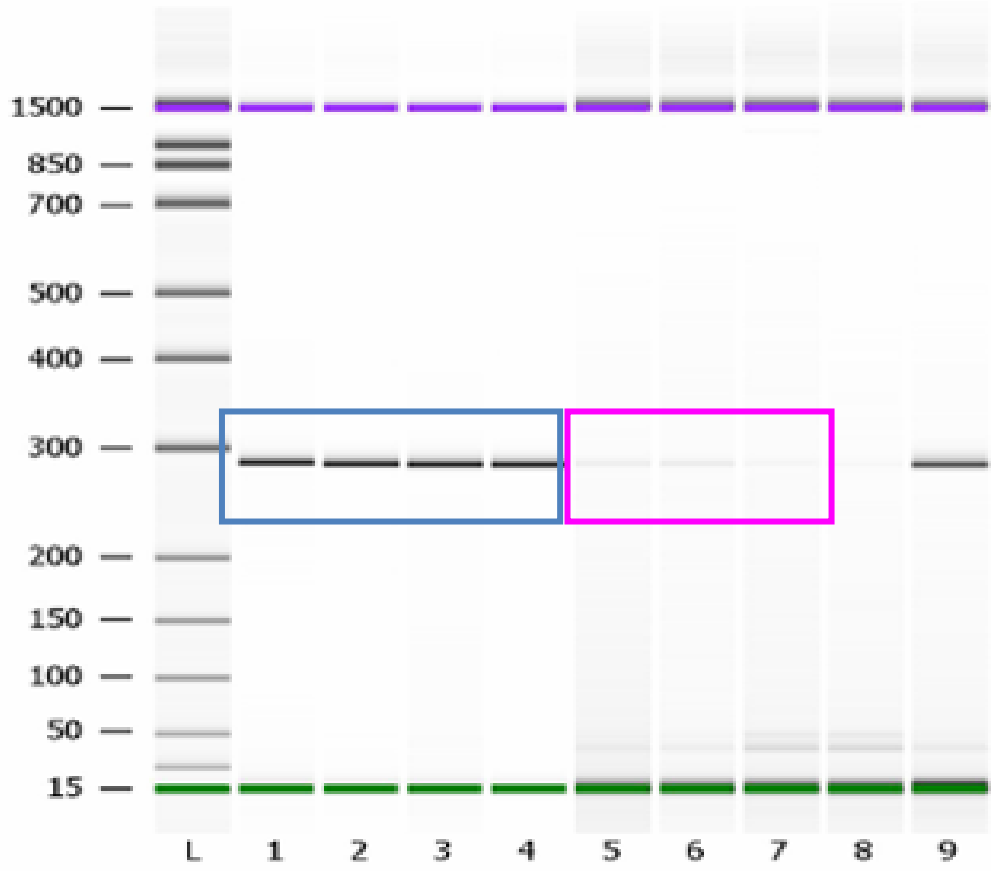
UNIVERSITY OF NORTH TEXAS
Center for Human IDENTIFICATION
Laboratory of Forensic Anthropology • Laboratory for Molecular Identification



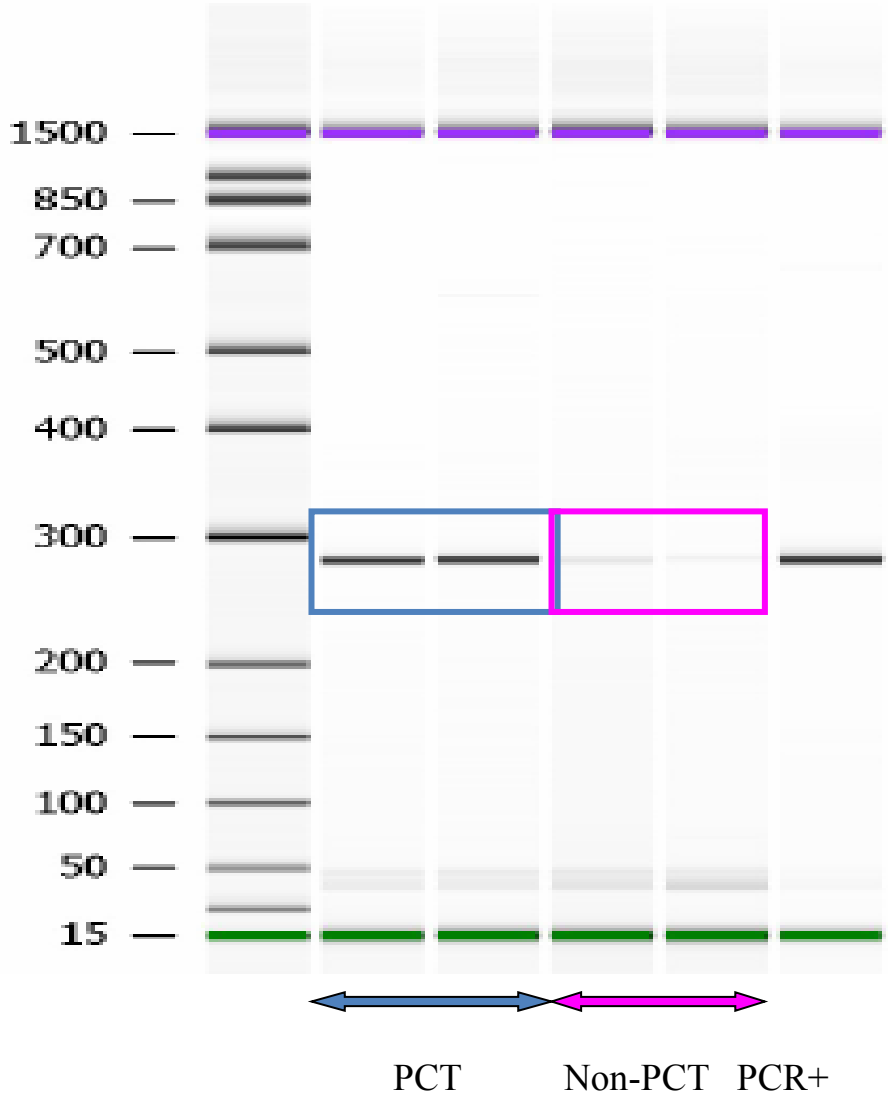
mtDNA from a Single Human Hair



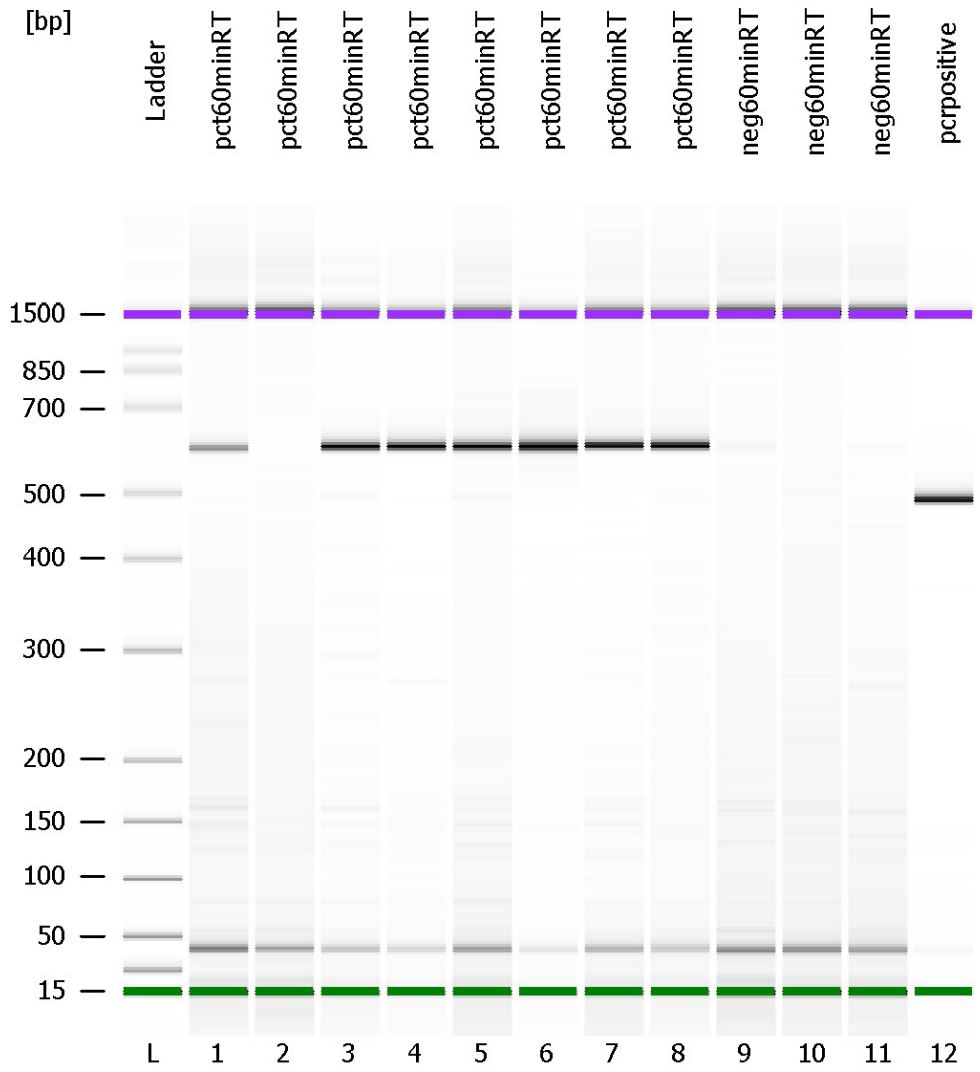
mtDNA from Human Skin Collected on Tape



mtDNA from Human Blood from a Single Fiber



Genomic DNA from Pig Bone



Acknowledgements

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Loreen Lofts
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Stephen Little