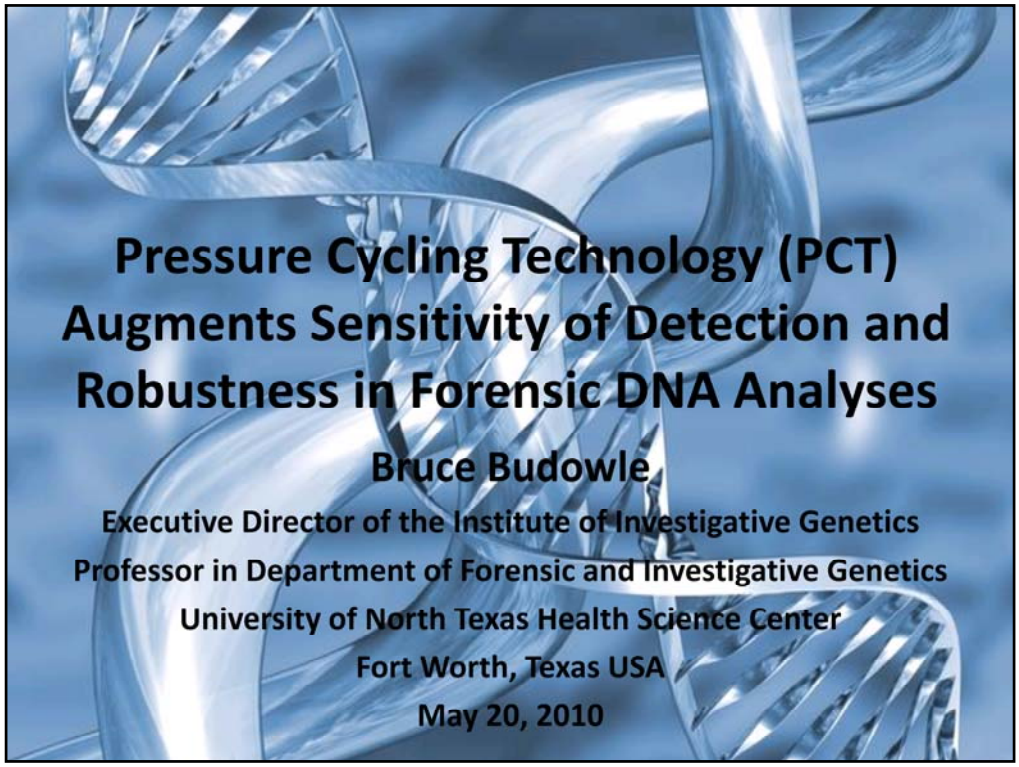
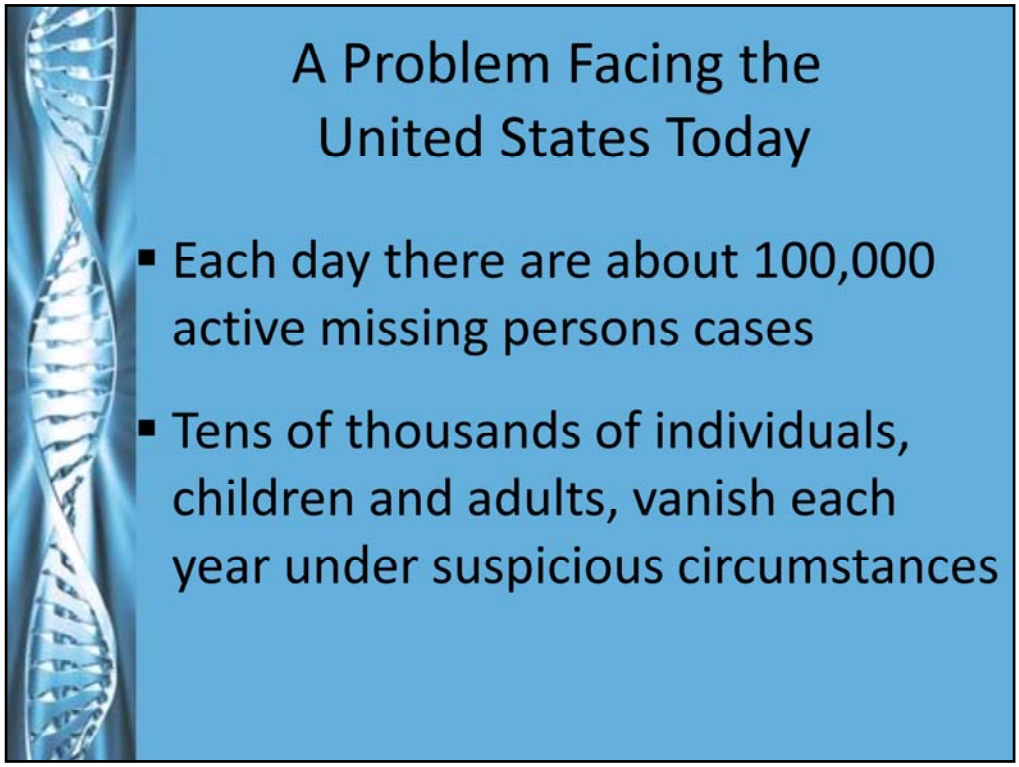
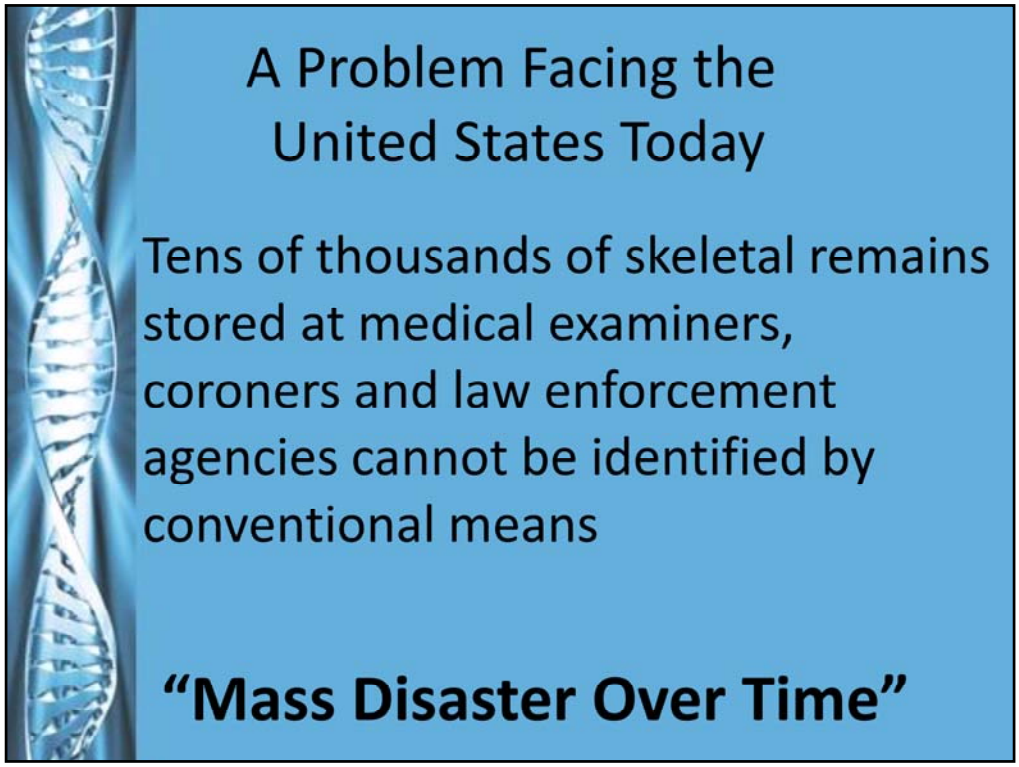


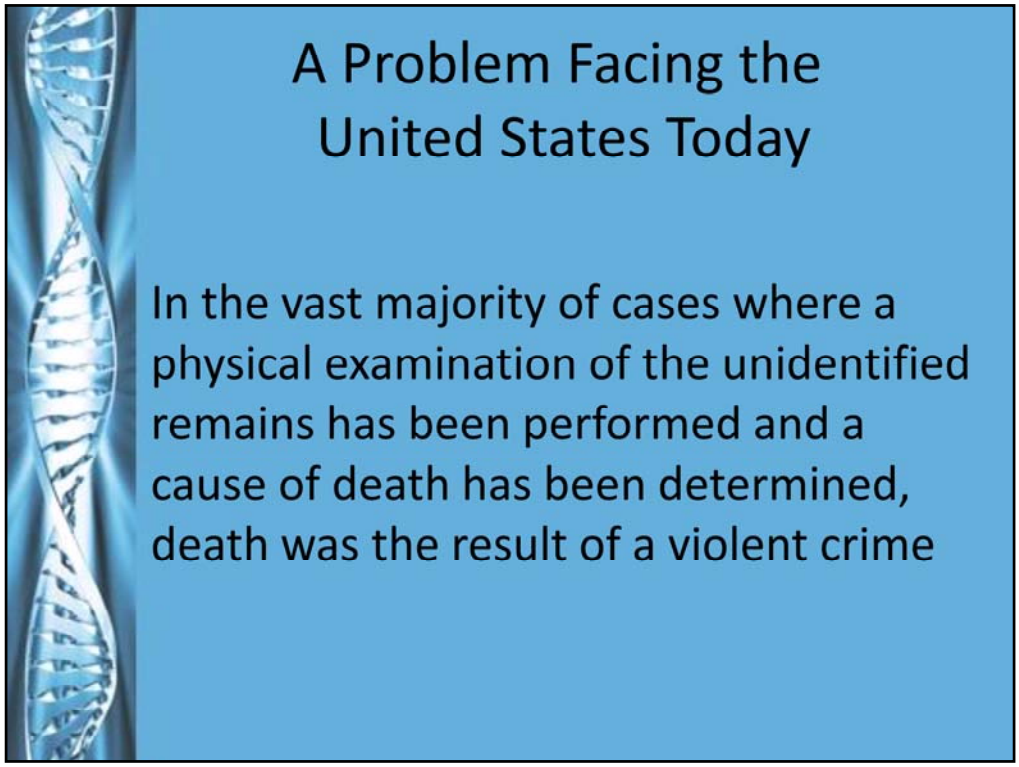
My portion of this study

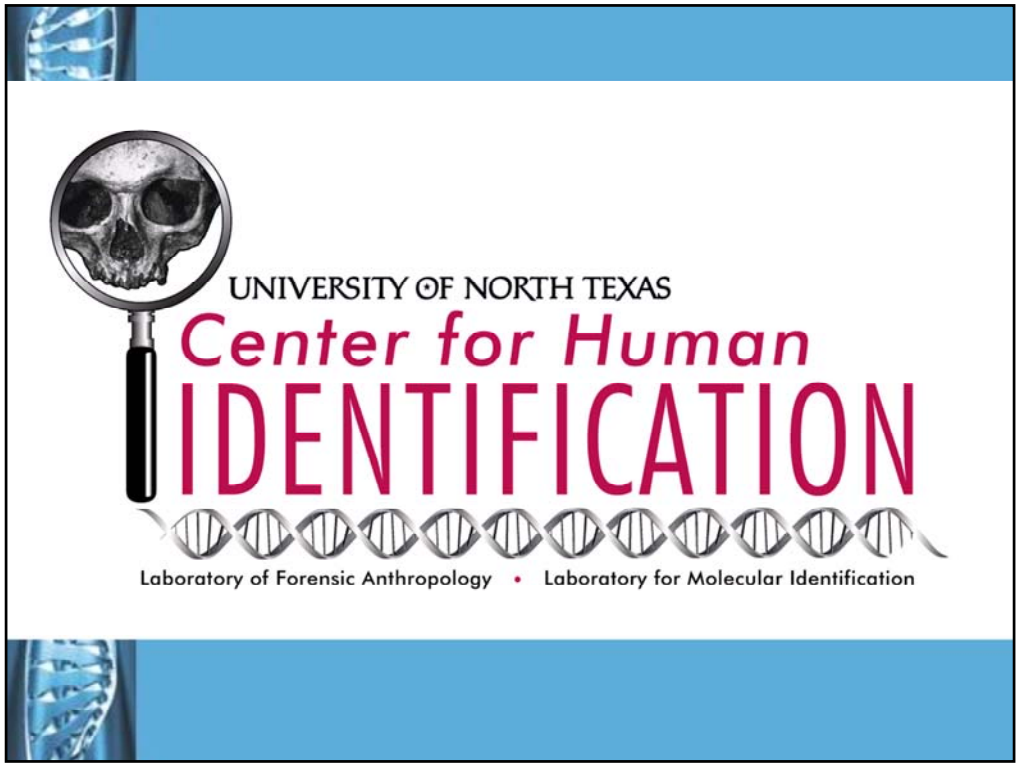
A Problem Facing the United States Today

Mass Disasters receive world-wide attention; however, within the United States, the magnitude of the problem involving unidentified human remains and missing persons is only beginning to receive attention


A Problem Facing the United States Today
Each day there are about 100,000 active missing persons cases
Tens of thousands of individuals, children and adults, vanish each year under suspicious circumstances


A Problem Facing the
United States Today
Tens of thousands of skeletal remains stored at medical examiners, coroners and law enforcement agencies cannot be identified by conventional means
"Mass Disaster Over Time"


A Problem Facing the
United States Today
In the vast majority of cases where a physical examination of the unidentified remains has been performed and a cause of death has been determined, death was the result of a violent crime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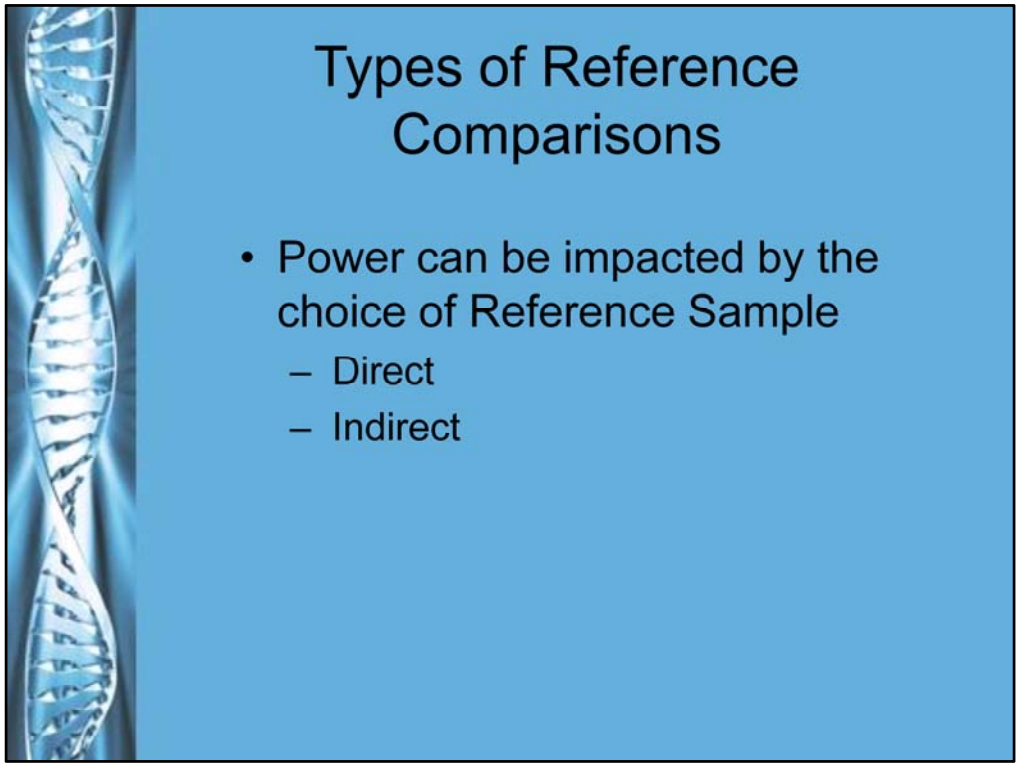
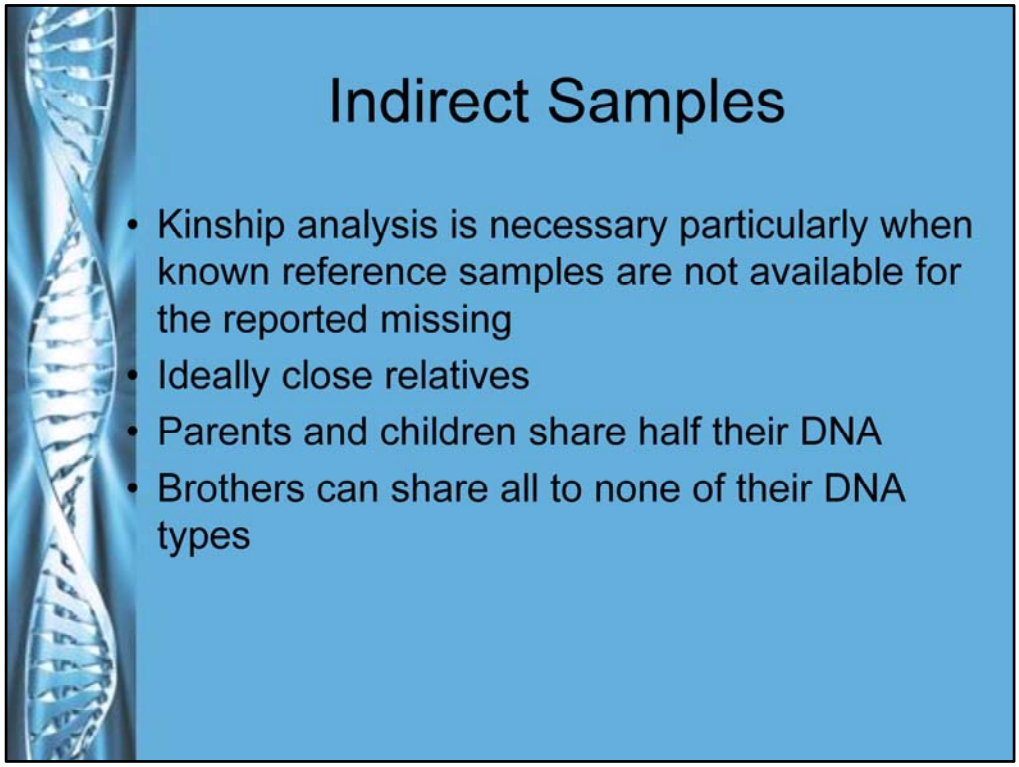
Center for Human
IDENTIFICATION
Laboratory of Forensic Anthropology • Laboratory for Molecular Identification

Missing Persons Identification
• Unidentified Human Remains
• Family References
• Kinship Analysis
• No direct comparisons


Types of Reference Comparisons
• Power can be impacted by the choice of Reference Sample
– Direct
– Indirect

Direct Samples


Indirect Samples
• Kinship analysis is necessary particularly when known reference samples are not available for the reported missing
• Ideally close relatives
• Parents and children share half their DNA
• Brothers can share all to none of their DNA types

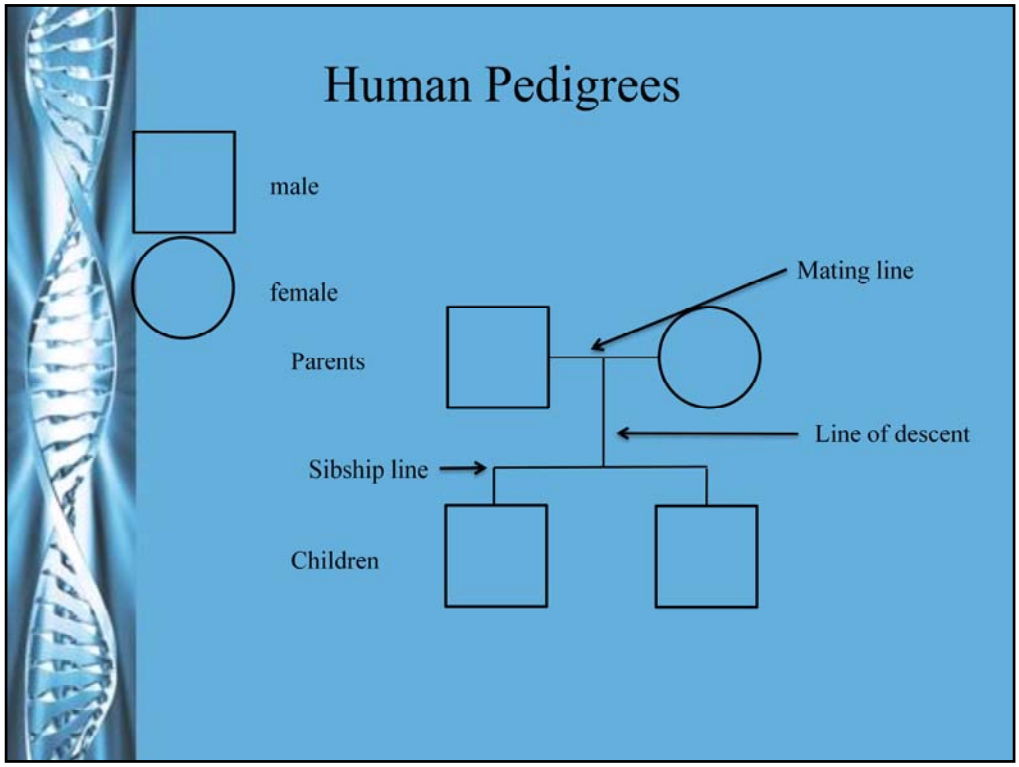


Figure 2.20 right

Challenged samples -
increase number of
genetic markers

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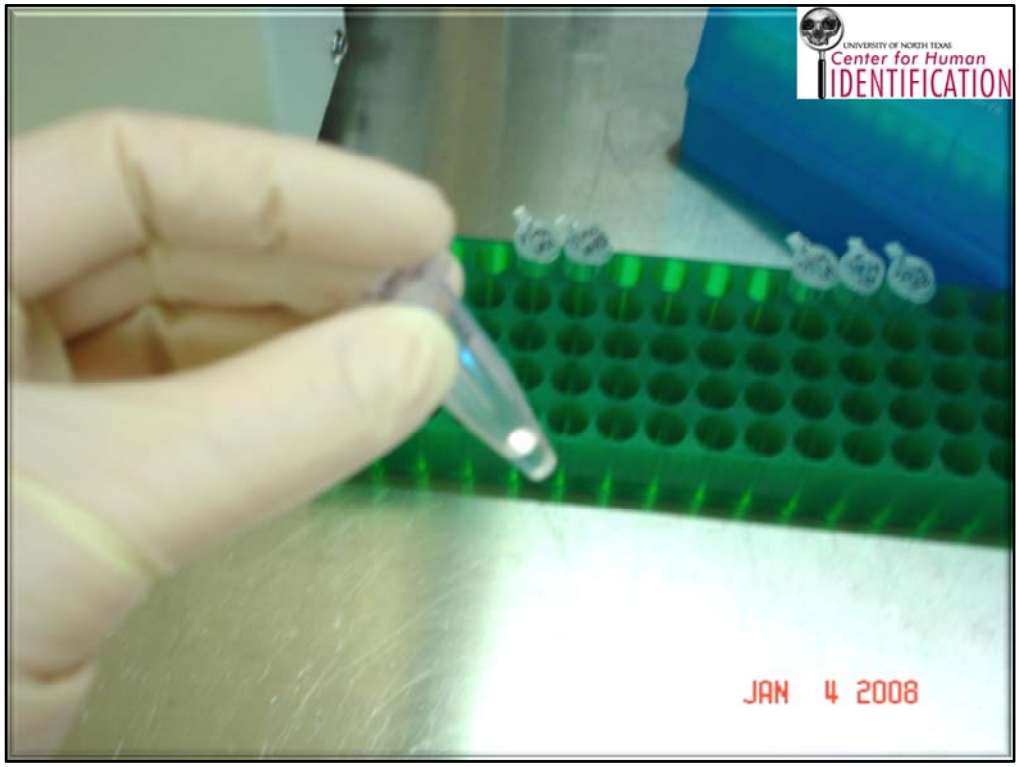
JAN 4 2008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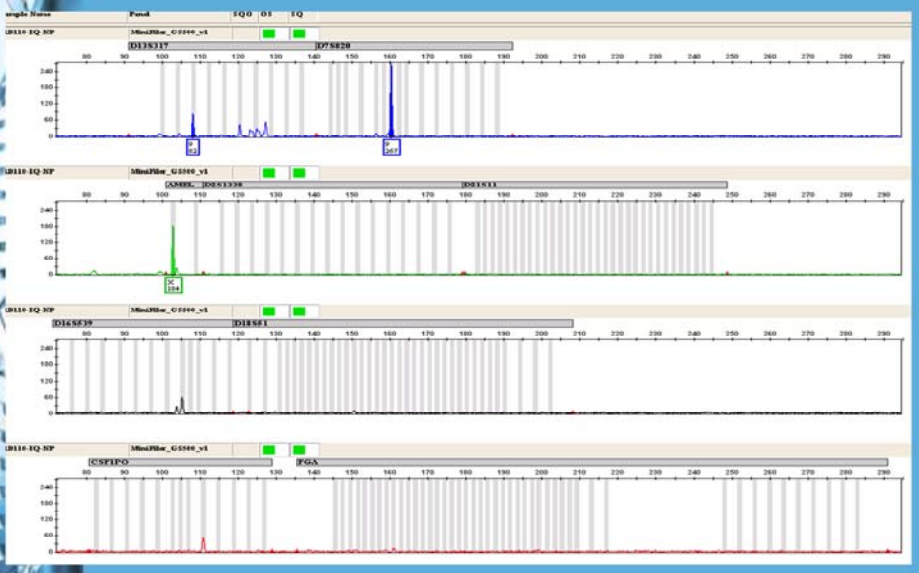
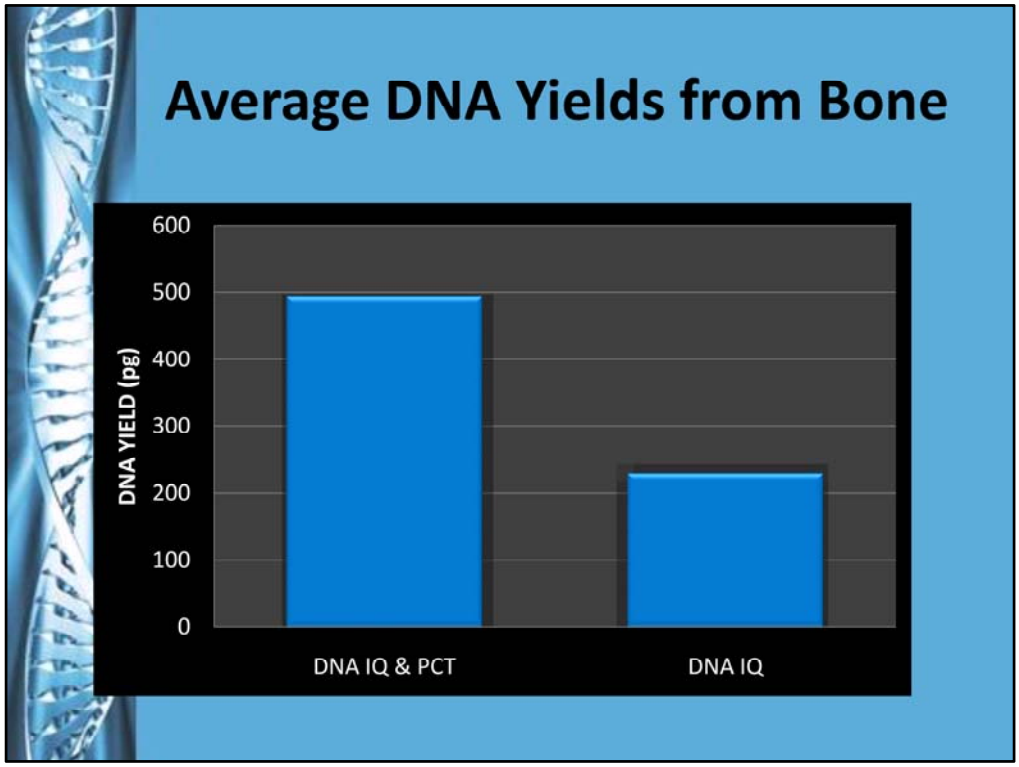
Center for Human
IDENTIFICATION
JAN 4 2008

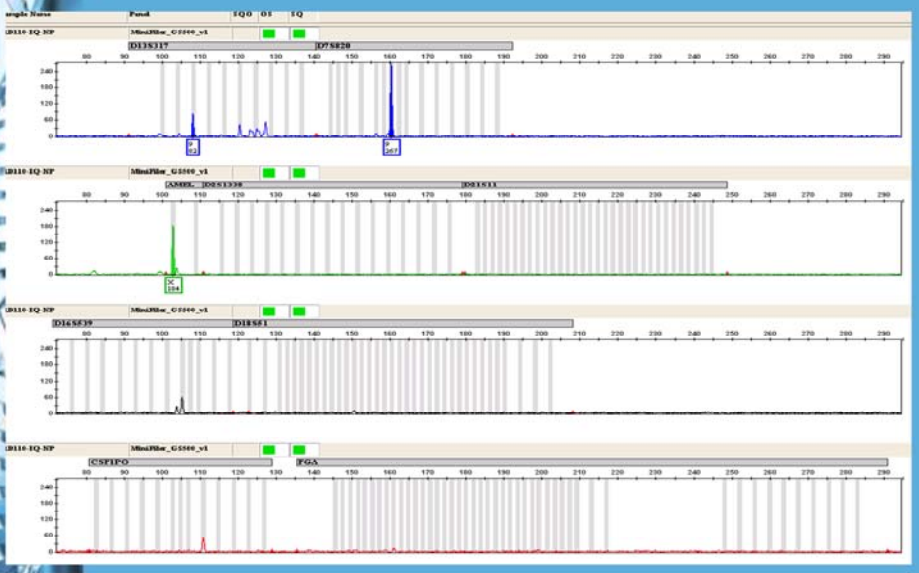
Bone Sample
(Low DNA Yield)




Average DNA Yields from Bone
DNA YIELD (pg)
600
500
400
300
200
100
0
DNA IQ & PCT
DNA IQ

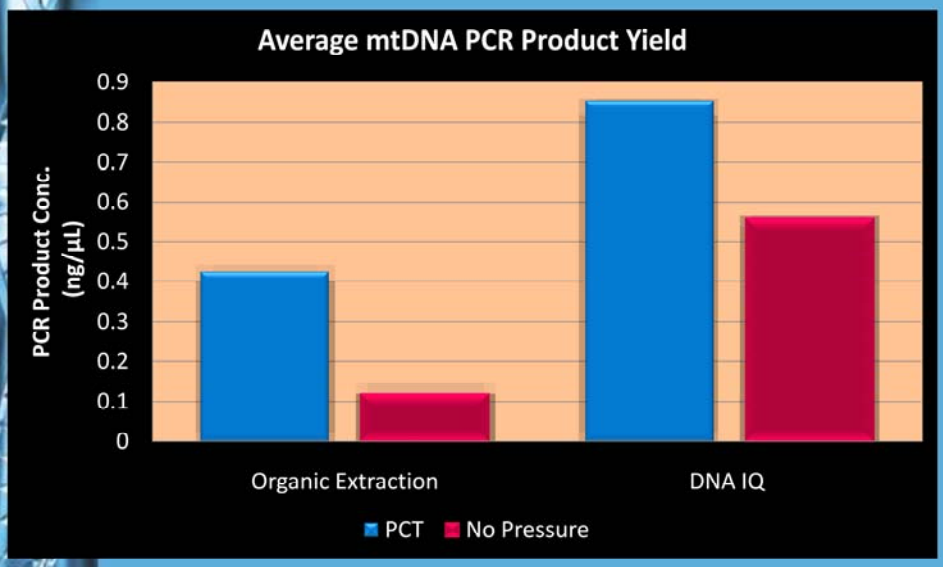
Bone Sample

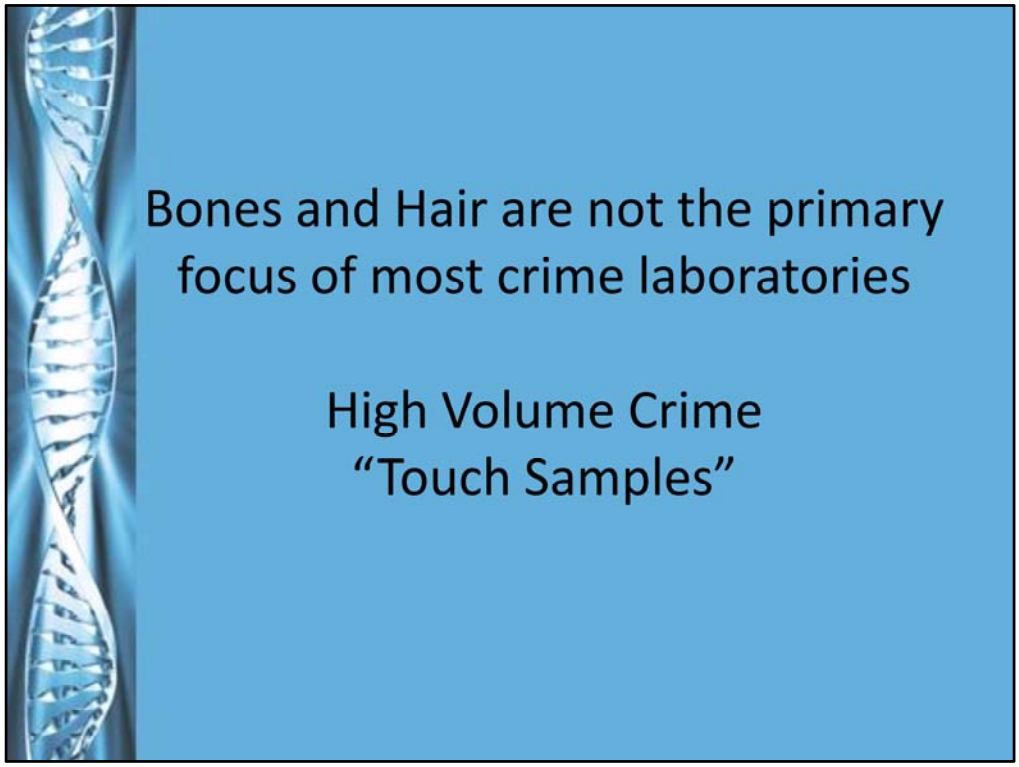
(No Pressure)



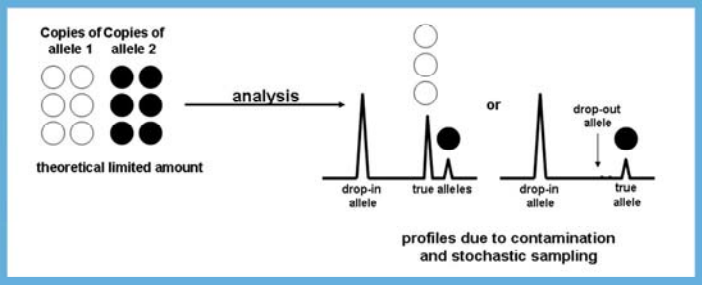
Bone Sample
(PCT)

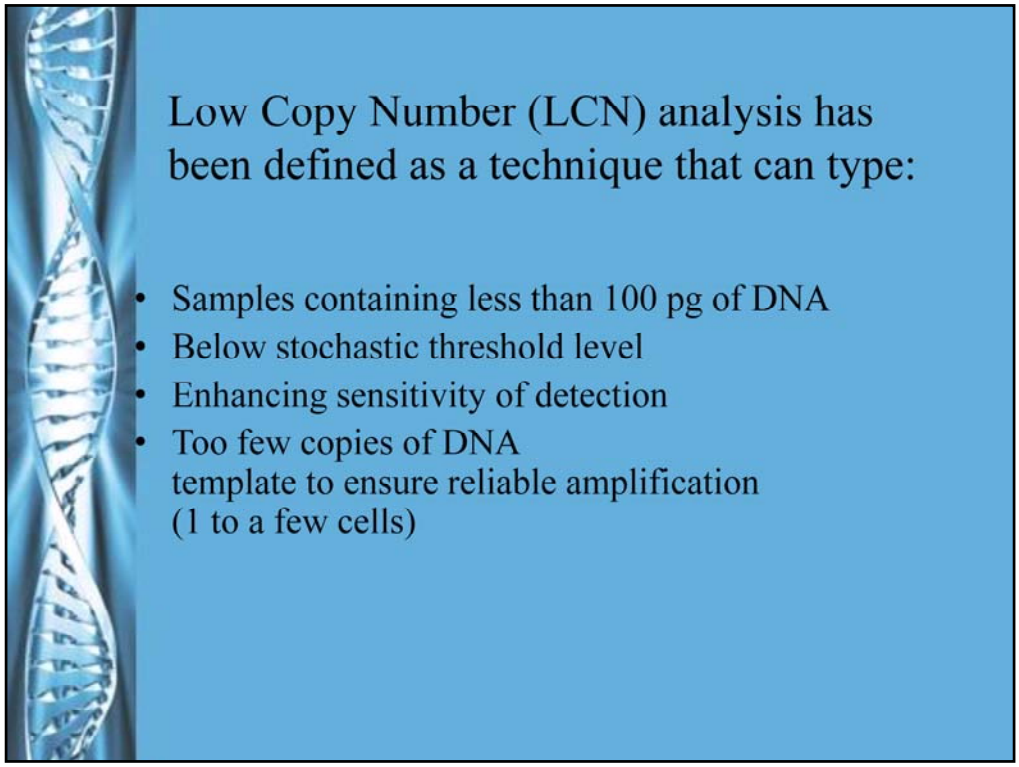
Hair Shafts

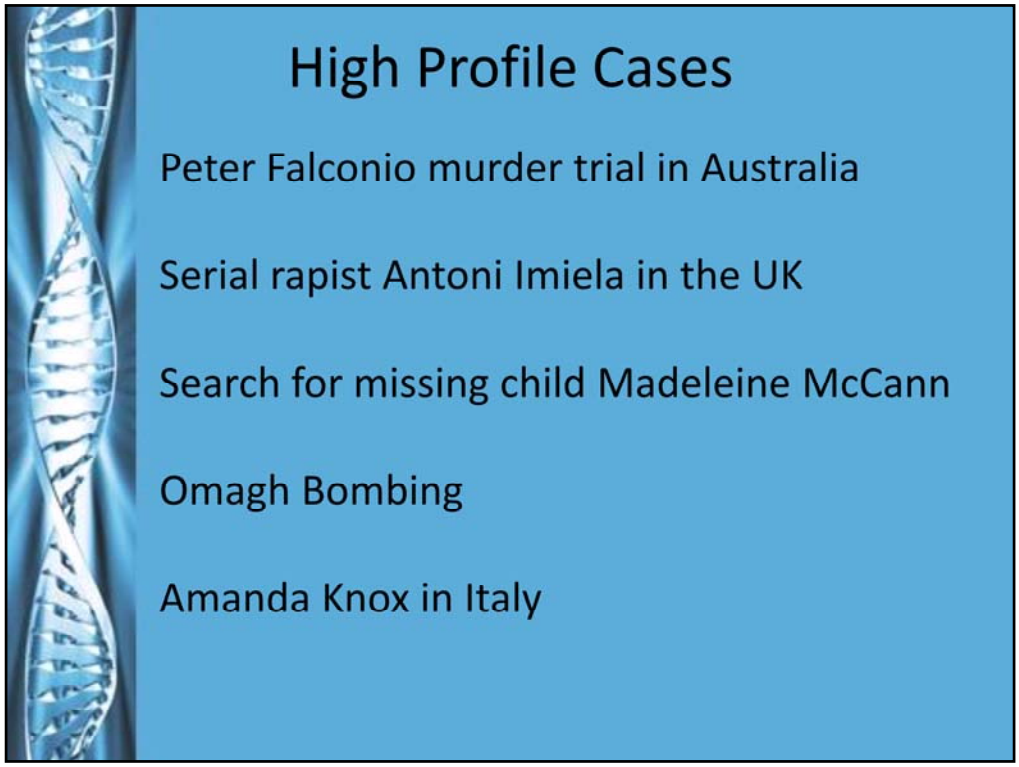

Average mtDNA PCR Product Yield
PCR Product Conc. (ng/µL)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Organic Extraction
DNA IQ
PCT
No Pressure

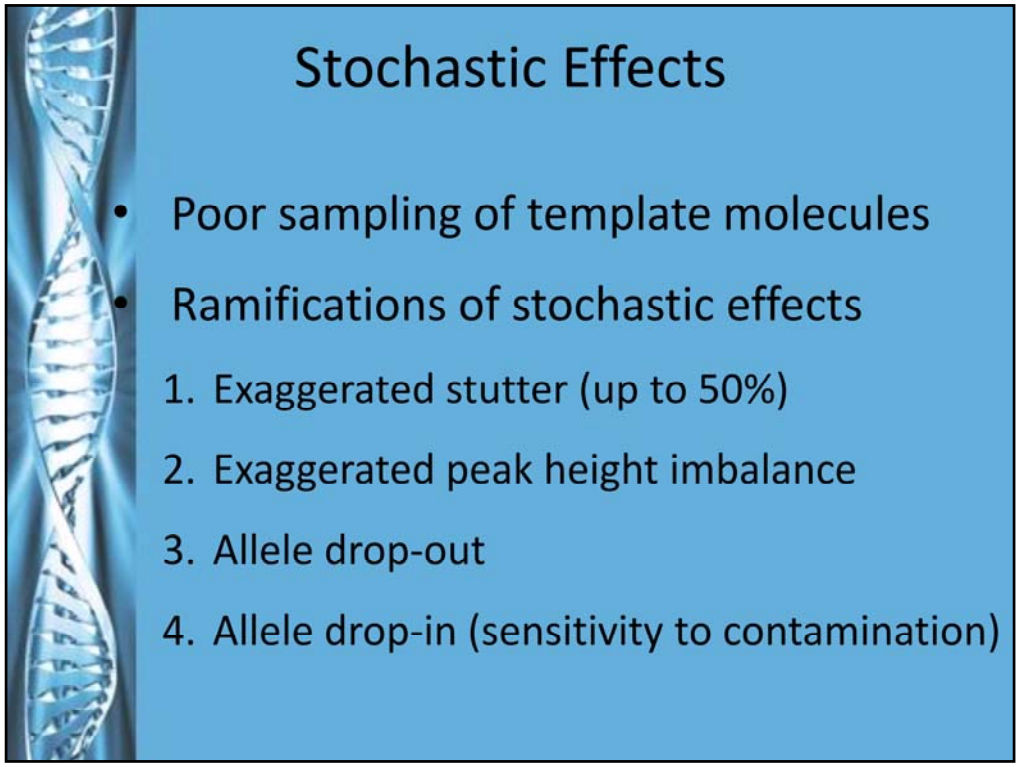

Bones and Hair are not the primary focus of most crime laboratories
High Volume Crime
"Touch Samples"

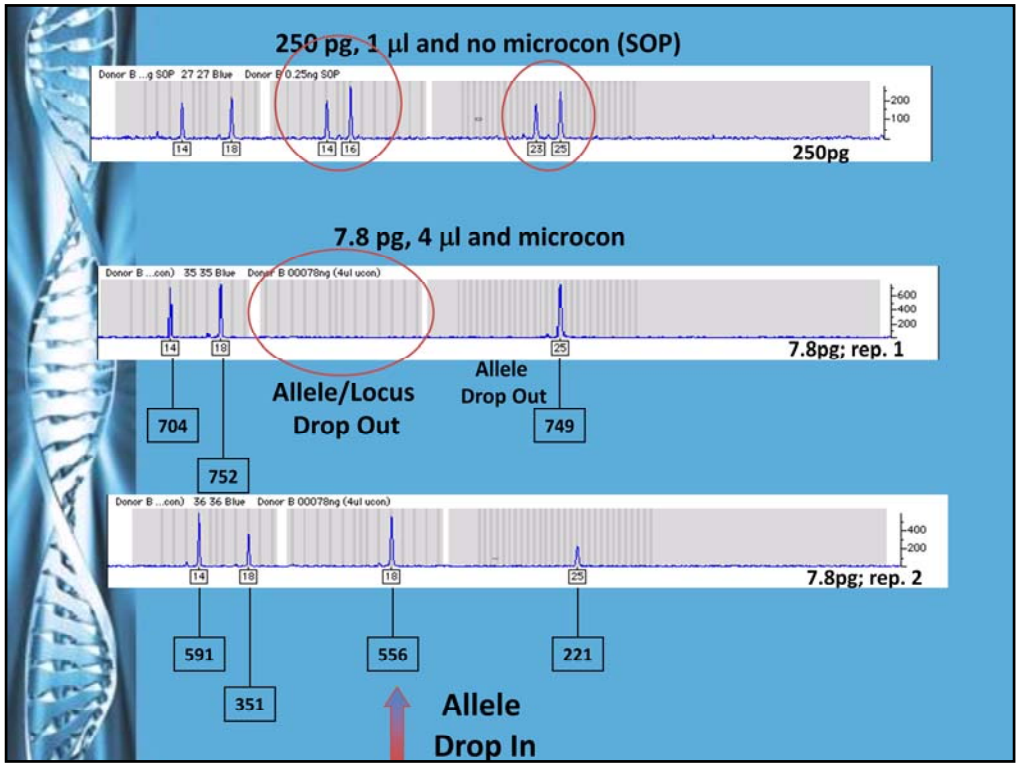
Low Copy Number Typing

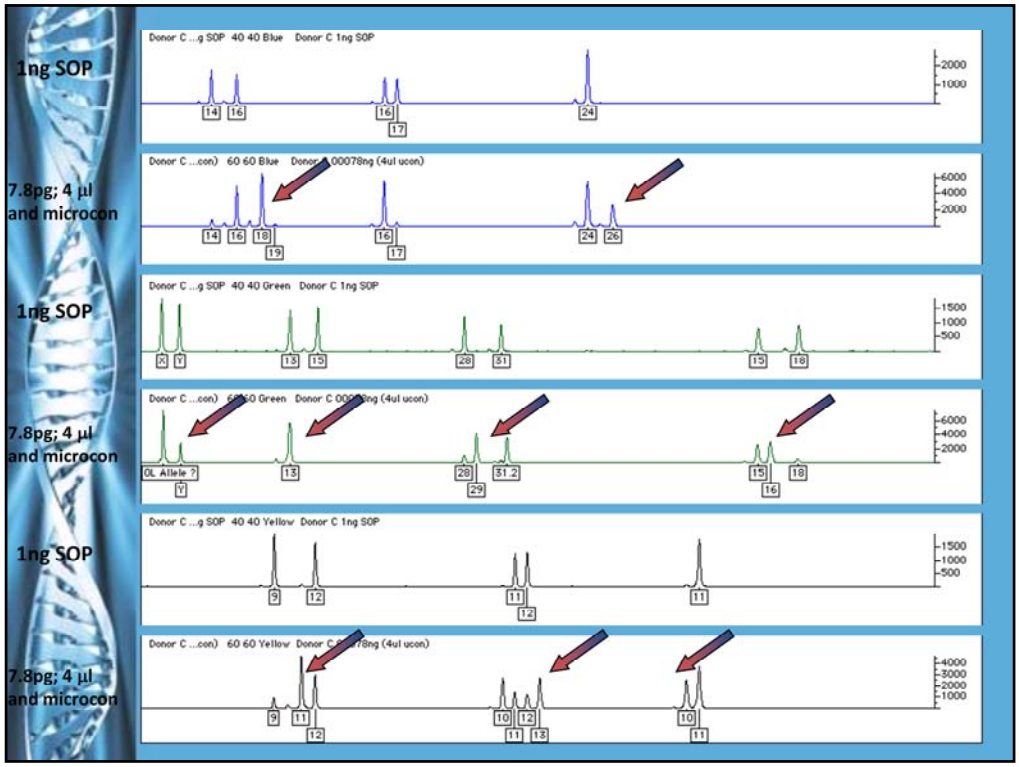



Low Copy Number (LCN) analysis has been defined as a technique that can type:
• Samples containing less than 100 pg of DNA
• Below stochastic threshold level
• Enhancing sensitivity of detection
• Too few copies of DNA template to ensure reliable amplification (1 to a few cells)

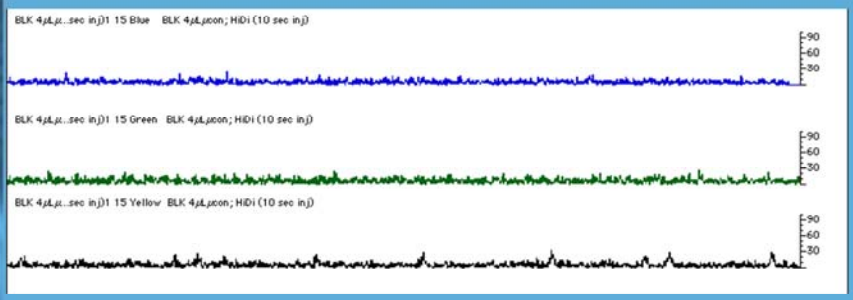

High Profile Cases
Peter Falconio murder trial in Australia
Serial rapist Antoni Imiela in the UK
Search for missing child Madeleine McCann
Omagh Bombing
Amanda Knox in Italy

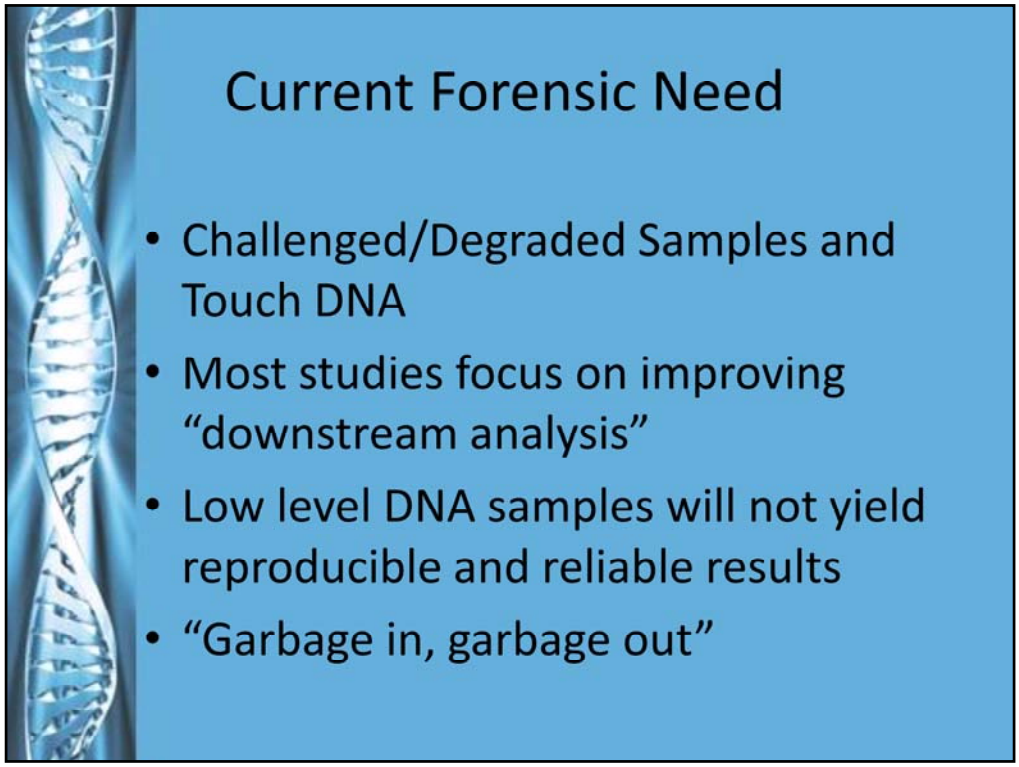

Stochastic Effects
• Poor sampling of template molecules
• Ramifications of stochastic effects
1. Exaggerated stutter (up to 50%)
2. Exaggerated peak height imbalance
3. Allele drop-out
4. Allele drop-in (sensitivity to contamination)


250 pg, 1 µl and no microcon (SOP)
250pg
7.8 pg, 4 µl and microcon
7.8pg; rep. 1
Allele/Locus Drop Out
Allele Drop Out
704
752
749
7.8pg; rep. 2
591
351
556
221
Allele Drop In


1ng SOP
7.8pg; 4 µl and microcon
1ng SOP
7.8pg; 4 µl and microcon
1ng SOP
7.8pg; 4 µl and microcon

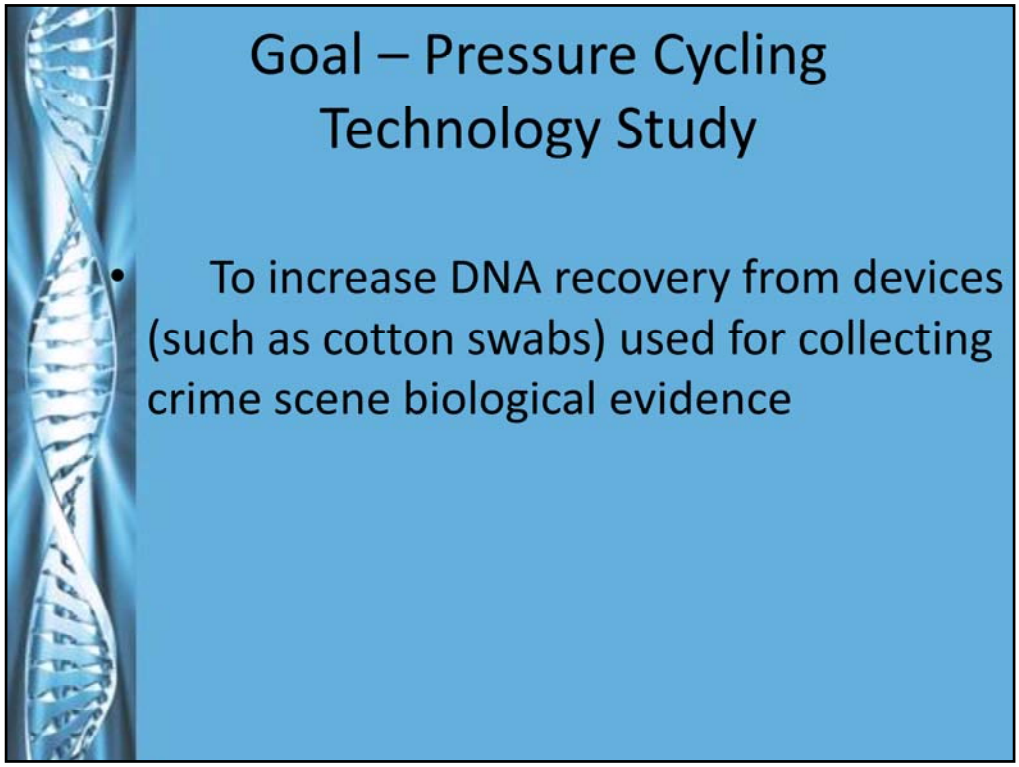
Amplification Blank
4µL amplicon,microcon, 10 second injection

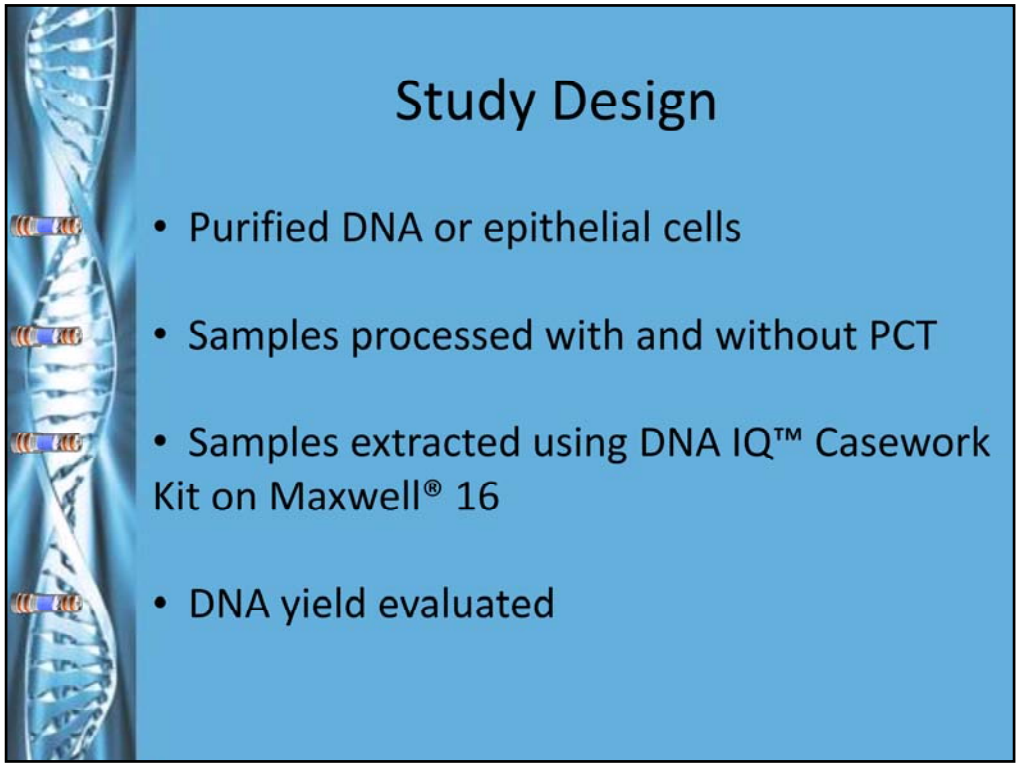




"Garbage in, garbage out"

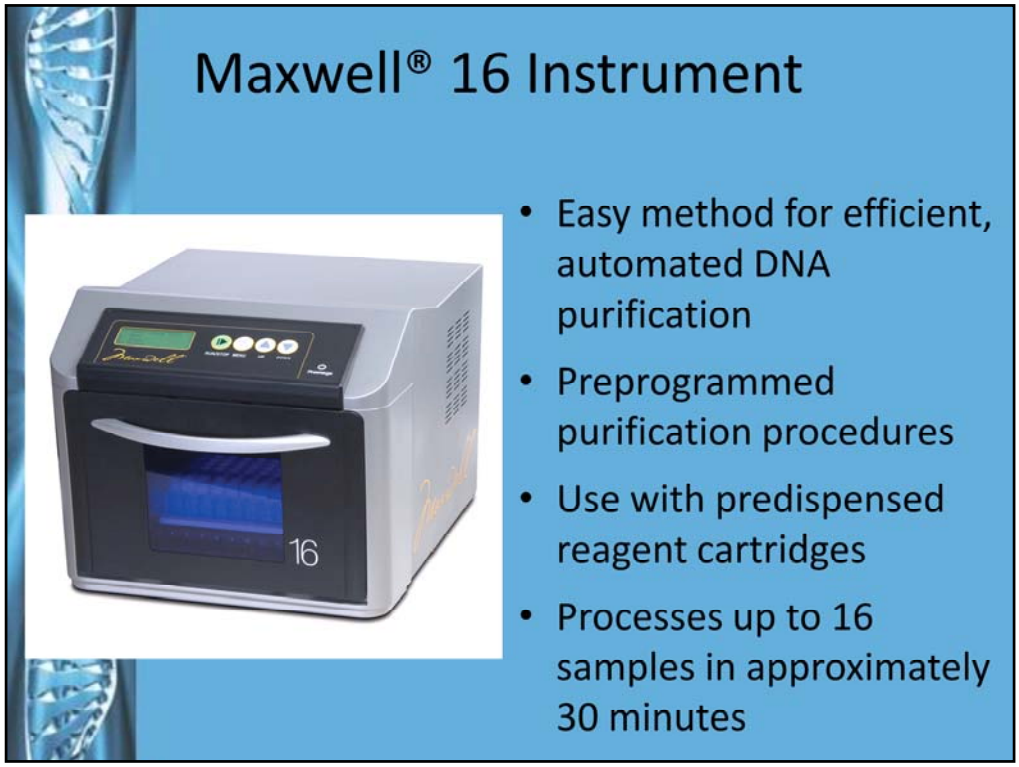
List steps where DNA could be lost (collection…extraction)


Goal – Pressure Cycling Technology Study
To increase DNA recovery from devices (such as cotton swabs) used for collecting crime scene biological evidence

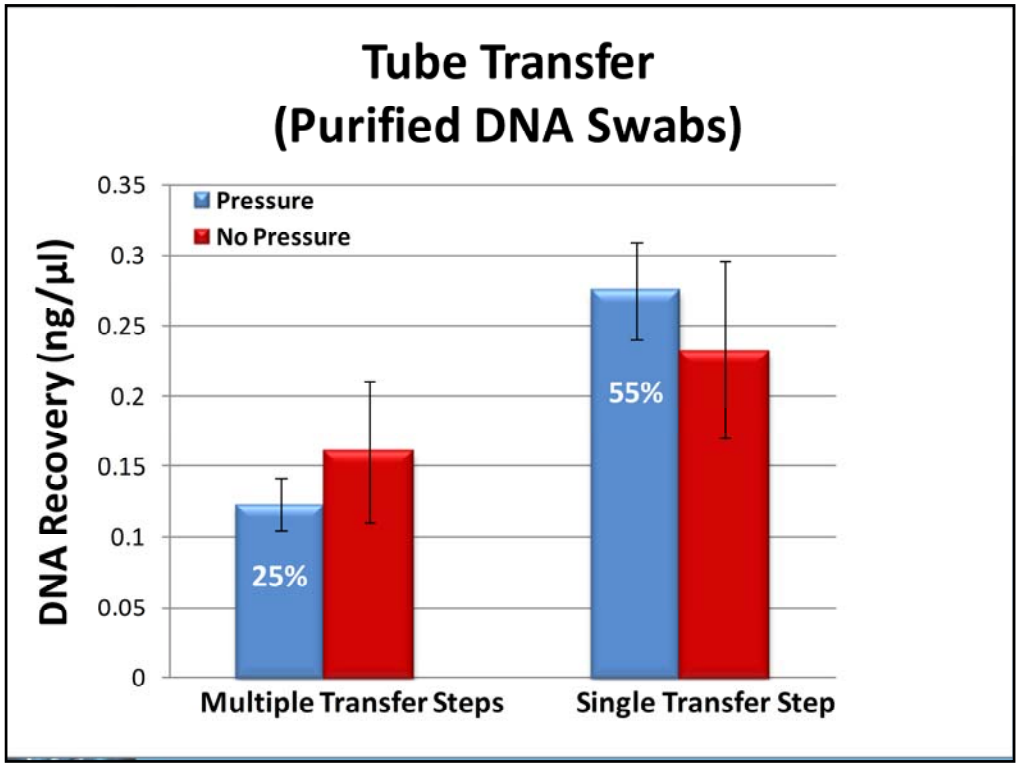


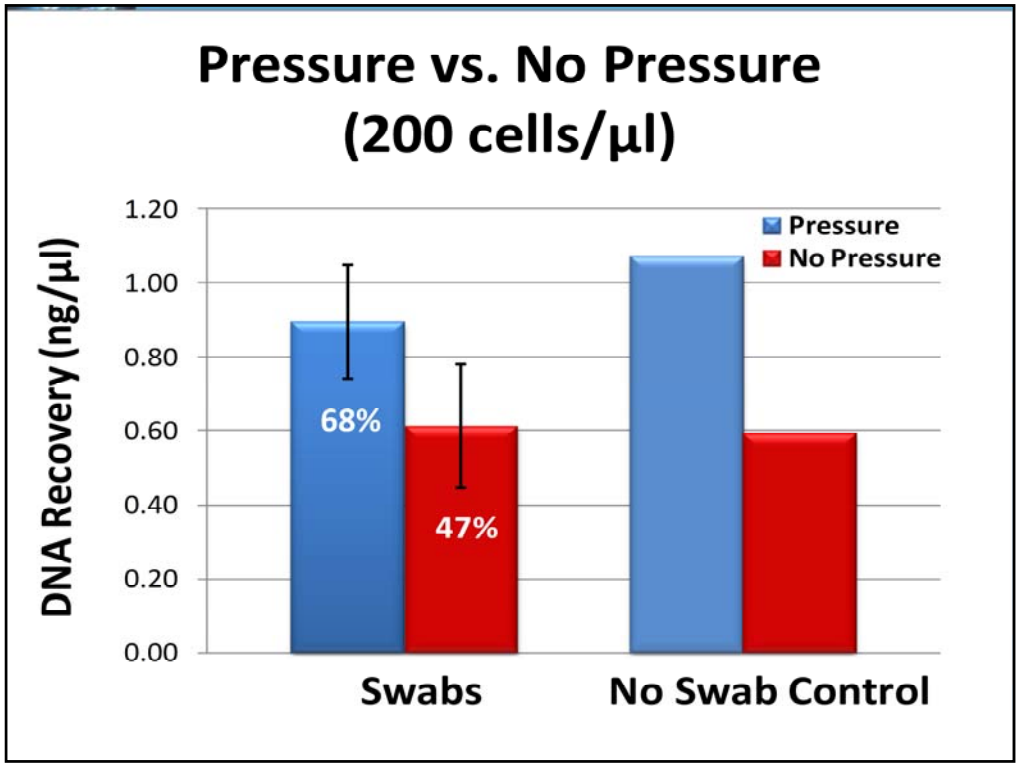
1:2 volume (250ul incubation buffer/500 lysis)

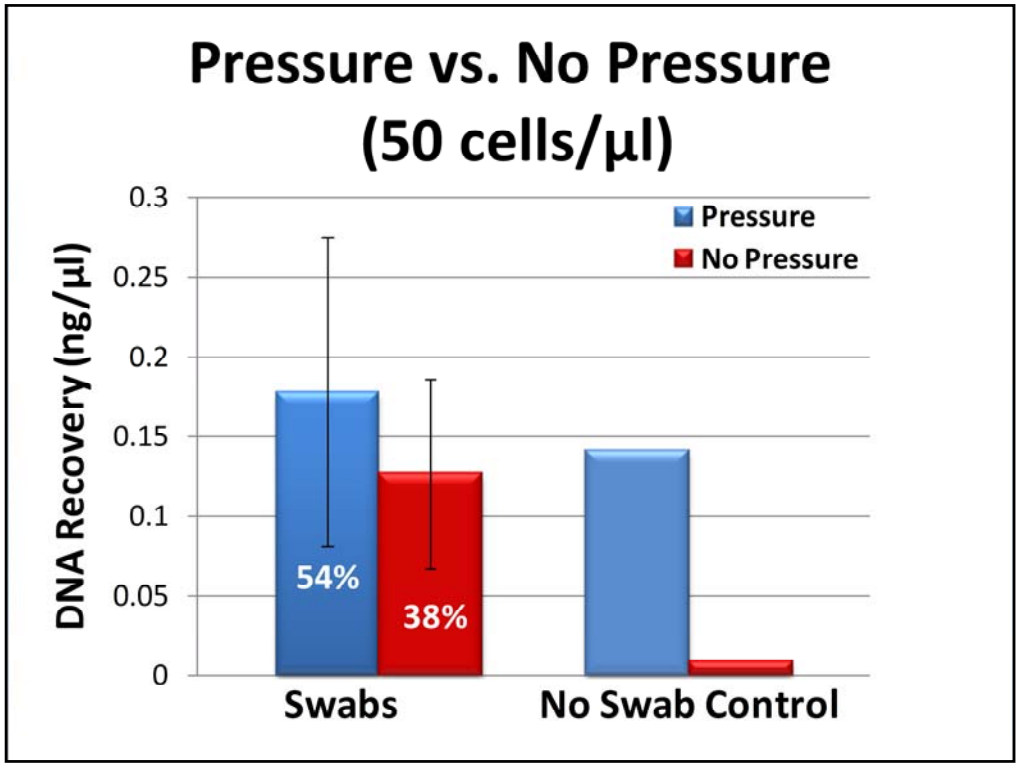
Max volume 750ul

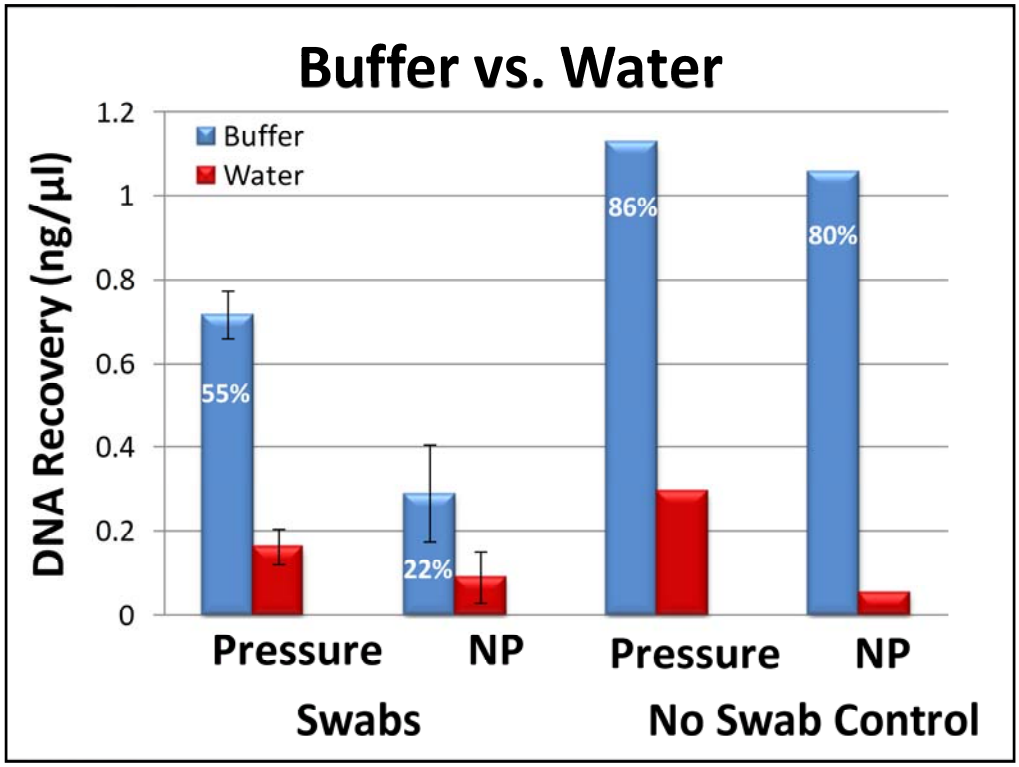


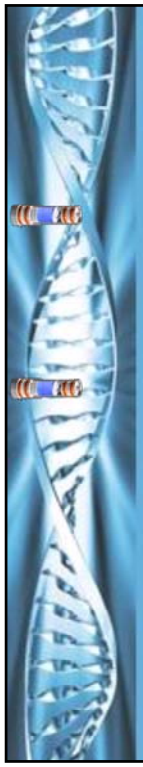
Convenient method for moving beyond PCIA extraction – health concerns, time


Tube Transfer
(Purified DNA Swabs)
DNA Recovery (ng/µl)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Pressure
No Pressure
25%
55%
Multiple Transfer Steps
Single Transfer Step


Pressure vs. No Pressure
(200 cells/µl)
DNA Recovery (ng/µl)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Pressure
No Pressure
68%
47%
Swabs
No Swab Control

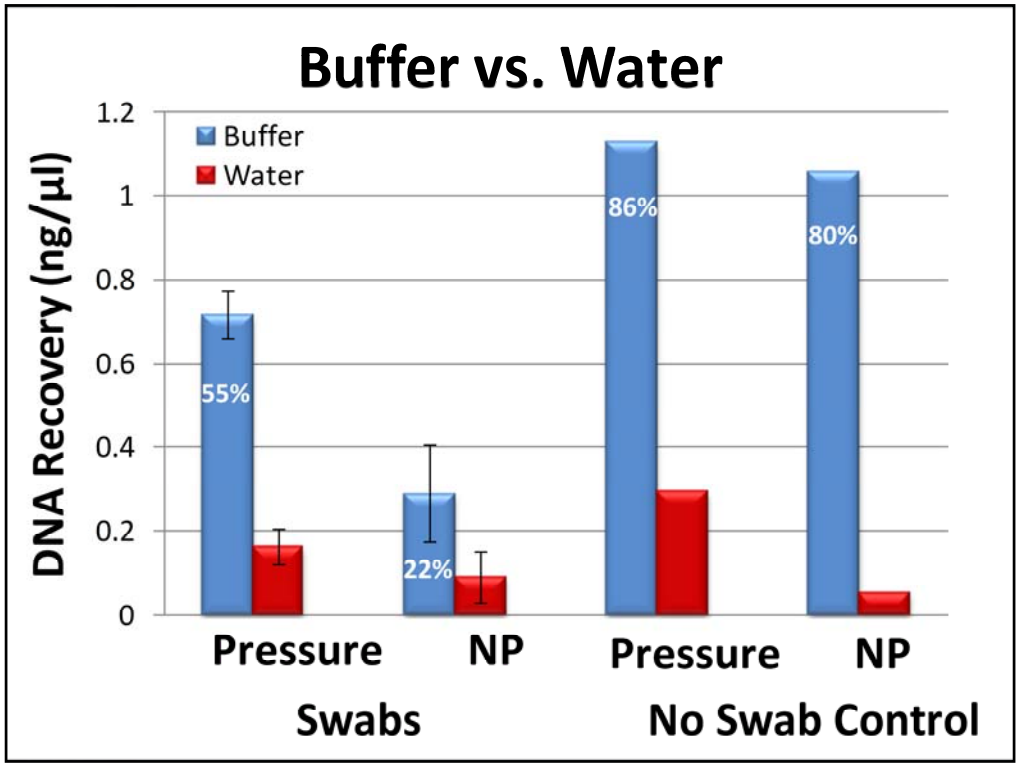

Pressure vs. No Pressure
(50 cells/µl)
DNA Recovery (ng/µl)
0.3
0.25
0.2
0.15
0.1
0.05
0
Pressure
No Pressure
54%
38%
Swabs
No Swab Control


Buffer vs. Water
DNA Recovery (ng/µl)
1.2
1
0.8
0.6
0.4
0.2
0
Buffer
Water
55%
22%
86%
80%
Pressure
NP
Pressure
NP
Swabs
No Swab Control

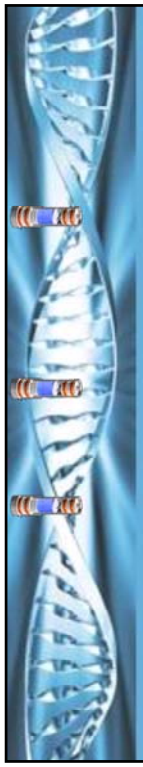


Additional Parameters Evaluated (Data Not Shown)

- Buffer Volumes (Incubation:Lysis Volumes – 1:1 vs. 1:2)
  - Results showed improved recovery with the 1:2 volume
- Tube siliconization
  - Results showed no difference in siliconized vs. non-siliconized PULSE tubes


Buffer vs. Water
DNA Recovery (ng/µl)
1.2
1
0.8
0.6
0.4
0.2
0
Buffer
Water
55%
22%
86%
80%
Pressure
NP
Pressure
NP
Swabs
No Swab Control

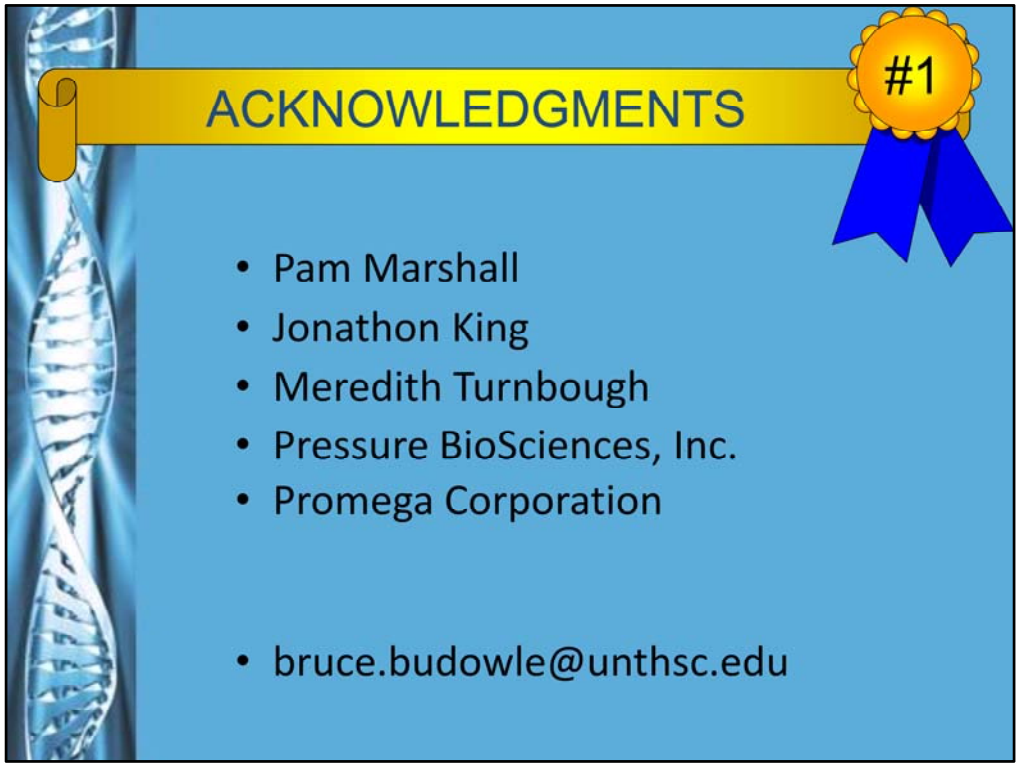
Blocking Agents



Summary

- Increased DNA yield in samples following PCT compared with those samples not exposed to pressure
- PCT can be used in conjunction with commercially available extraction reagents
- Enhance robustness (less stochastic effects)
- More cases potentially analyzed
- Results are still variable
- Need to address blocking of inner surface

Future Directions
• Alternate Collection Devices
• Vary Extraction Methods
• Blocking Agents


ACKNOWLEDGMENTS
#1
• Pam Marshall
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• Promega Corporation
• bruce.budowle@unthsc.edu