Pressure BioSciences, Inc.

PBI Shredder Systems

Gentle, Rapid, and Safe Disruption of Tissues and Organisms for the Extraction of DNA, RNA, Protein, Mitochondria, Lipid, and Small Molecules

The PCT Shredder or The SHREDDER SG3

Discovery Starts with Sample Preparation™

Barocycler®, PULSE®Tubes, *The PCT Shredder*™, *The* SHREDDER SG3™
PCT Shredder PULSE Tubes™ and Shredder Tubes™
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PBI Shredder Systems are low shear mechanical devices for gentle, rapid, and safe disruption of tissues and organisms. Both *The PCT Shredder* and *The* SHREDDER SG3 can provide effective extraction of DNA, RNA, proteins, mitochondria, lipids and small molecules from tissues and organisms, when used with Shredder Tubes and various buffers.

Both systems use a variety of Shredder Tubes to directly and rapidly grind solid samples providing easy handling and reducing sample contamination. And, although not required for all extractions, additional extraction efficiency can be achieved when this process is combined with pressure cycling technology (PCT).

PBI SHREDDER SYSTEMS

The PCT Shredder

Features and Benefits *of The PCT Shredder*

- Portable
- Sturdy, Stainless Steel Construction
- Cordless
- Compact and Lightweight driver

Features and Benefits of *The* SHREDDER SG3

- Long Lasting Lithium Rechargeable Batteries
- Heavy Duty Robust Driver
- Three Position Lever for Setting Reproducible Force

***The* SHREDDER SG3**

SHREDDER TUBES

Features & Benefits of the Shredder Tube:

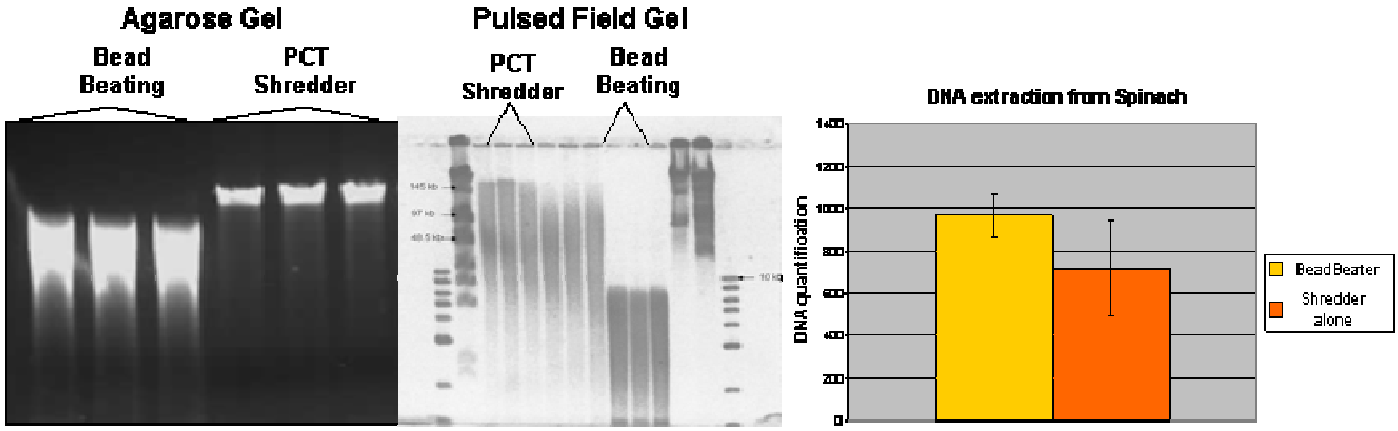
- A Variety of Single-Use Processing Containers
- Standard Tubes for Ambient Pressure Processing
- Specialized Tubes for Ultra-High Pressure Processing
- Metal Inserts for the Toughest of Samples
- Excellent for Collection, Storage, Transport & Processing
- Closed Containers to Help Ensure Safety Throughout the Entire Sample Preparation Process

Variations and Specifications of Shredder Tubes

Tube Type	Color	Metal Disk	PCT Compatible
PCT Shredder PULSE Tubes FT500-S	White	No	Yes
PCT Shredder PULSE Tubes FT500-MS	White	Yes	Yes
Shredder Tubes FT500-PS	Coral	No	No
Shredder Tubes FT500-PMS	Coral	Yes	No

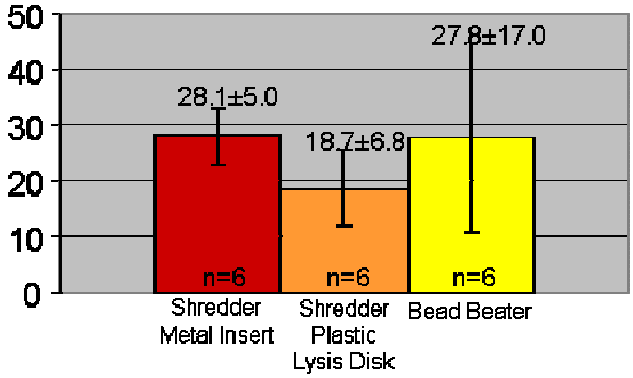
EXTRACTION OF DNA

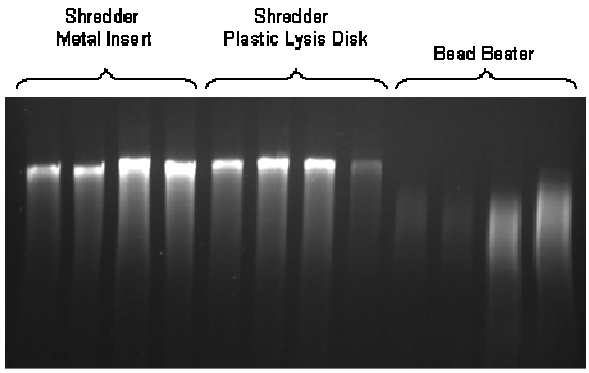
DNA from Spinach Shown on a Pulsed Field Gel



DNA Extraction from spinach using the PBI Shredder System Results in Much Longer DNA than bead beating methods, while providing comparable yields.

DNA Extraction from Apple Seeds





Consistent high yields of DNA can be obtained from apple seeds using either PBI Shredder System.

Shredder Tubes (FT500-PMS) or PCT Shredder PULSE Tubes (FT500-MS) with metal inserts give more consistent and higher recovery of DNA from hard samples, such as apple seeds.

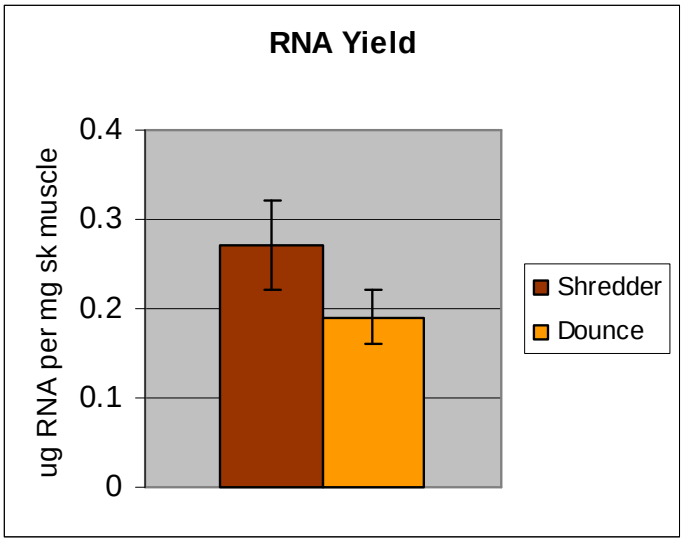
Agarose gel electrophoresis shows that PBI Shredder Systems yield higher molecular weight genomic DNA, while the bead beater causes significant shearing of DNA.

EXTRACTION OF RNA

RNA from Rat Skeletal Muscle

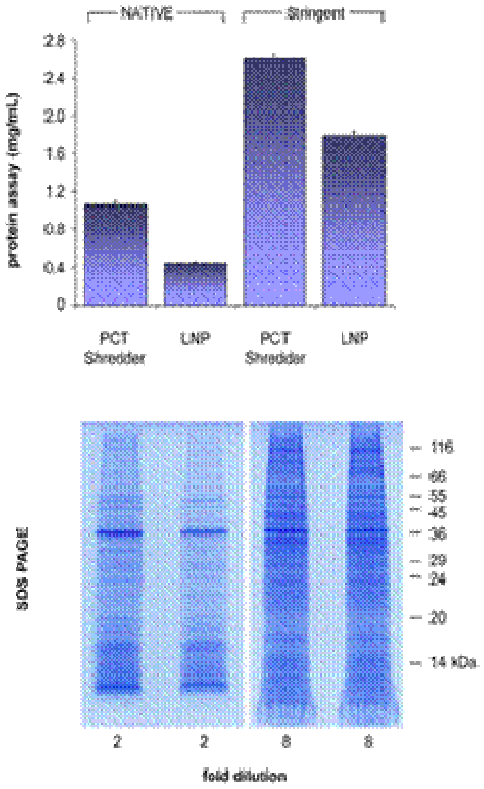
High quality RNA suitable for the preparation of cDNA libraries can be obtained with *The PCT Shredder* or *The* SHREDDER SG3.

Shredder Tubes are compatible with a wide variety of sample preparation kits and reagents including commercially available chaotropes for the extraction of nucleic acids.



EXTRACTION OF PROTEIN

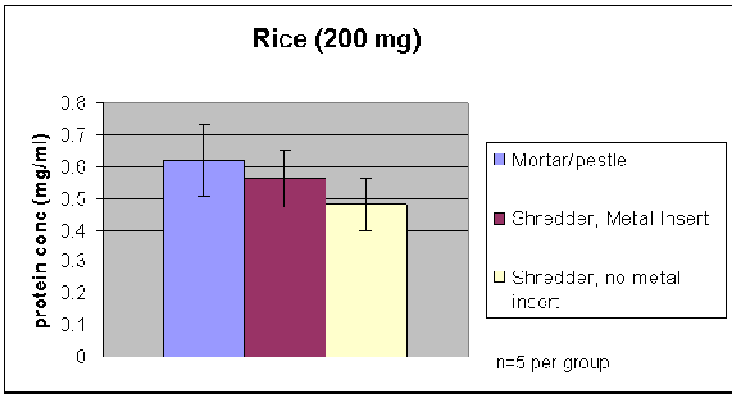
Protein from the Nematode C. elegans



Extraction of protein from *C. elegans* using *The PCT Shredder* and ProteoSolve-CE Native or ProteoSolve-CE Stringent Kits from PBI results in greater yield of protein than extraction with mortar and pestle with liquid nitrogen (LNP).

PBI's ProteoSolve-CE Native Kit was developed for the efficient recovery of native proteins and protein complexes under minimally denaturing conditions in which native conformation and activity are preserved.

Protein from Rice Grains

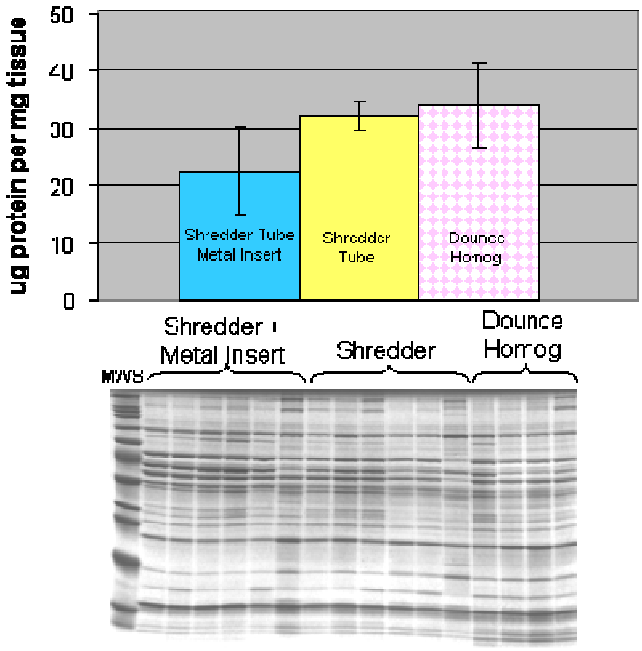


Although PBI Shredder Systems are comparable in yield to mortar and pestle methods, they are far less labor intensive than manual techniques.

With PBI Shredder Systems there is no need to transfer the ground material for further processing.

Since Shredder Tubes are single-use disposables, cleaning between extractions is no longer necessary.

Protein from Rat Skeletal Muscle

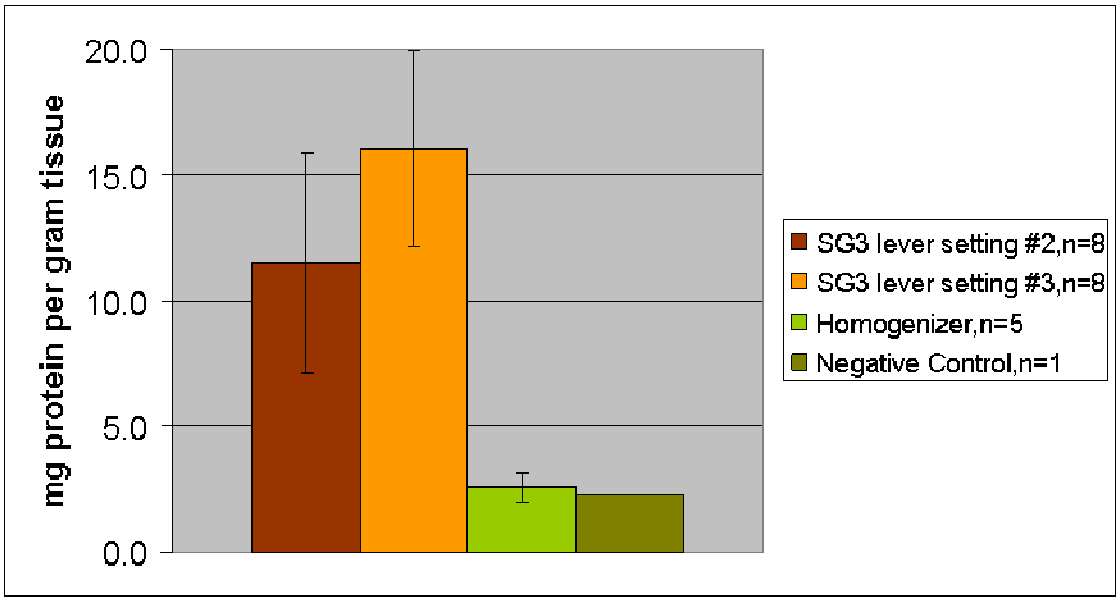


Consistent high yields of protein can be obtained from muscle tissue using PBI Shredder Systems.

Protein yield data demonstrate that Dounce homogenization is less consistent (large error bar), while the Shredder samples are grouped much more tightly (small error bar), indicating greater reproducibility.

Note that better results may be obtained for some sample types using PCT Shredder PULSE Tubes (FT500-S) or Shredder Tubes (FT500-PS) without metal inserts. This may be due to the sticky nature of some samples, which can clog the smaller pores of the metal insert.

Protein from Porcine Cartilage



Protein from porcine cartilage (~30 mg per sample) extracted in PBI ProteoSolve-IEF Buffer.

Tissue disruption was performed with *The* SHREDDER SG3 in PCT Shredder PULSE Tubes (FT500-MS) for 40 seconds at lever setting #2 or #3.

Comparison samples were disrupted using a commercially available homogenizer for 1 minute.

The negative control was generated by soaking a piece of cartilage in PBI ProteoSolve-IEF Buffer without mechanical disruption.

PROTEIN EXTRACTION WITHOUT LIQUID NITROGEN

TUMOR PROTEIN: PBI SHREDDER VS. CRYOGENIC GRINDING

Sample Type	Target Protein	Cryogenic Grinding Yield	PCT Shredder Yield
Metastatic Ovarian Tumor to The Omentum	FAS Ligand (a Single Transmembrane Segment Protein)	3±0.5 pg/mg Tissue	4.0±0.05 pg/mg Tissue
Fresh Frozen Prostate Cancer Tissue	Prostate-specific Membrane Antigen (a Single Transmembrane Segment 100K Protein)	~80% Recovery	~80% Recovery
Metastatic Ovarian Tumor to The Omentum	AKT1 (a Soluble Protein)	0.24 ng/mg Tissue	0.35 ng/mg Tissue

The PBI Shredder System can effectively replace liquid nitrogen grinding in some applications

Cryogenic grinding (mortar and pestle with liquid nitrogen) followed by extraction with PBI's ProteoSolve-TD2 Kit and pressure cycling technology (PCT) yielded less protein than disruption by *The* PBI Shredder System followed by extraction with PBI's ProteoSolve-TD2 Kit and PCT.

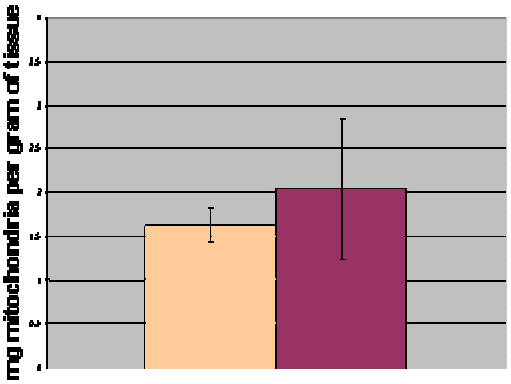
ProteoSolve-TD2 Buffer in combination with PCT facilitates recovery of intact membrane proteins from solid tumors. ProteoSolve-TD2 Buffer contains no organic solvents and is directly compatible with most subsequent proteomic analyses (2-D gels, SDS PAGE, liquid chromatography, antibody assays, and mass spectrometry).

ProteoSolve-TD2 Buffer also recovers soluble cytosolic proteins to provide a more complete representation of the cellular proteome than can be achieved by other methods.

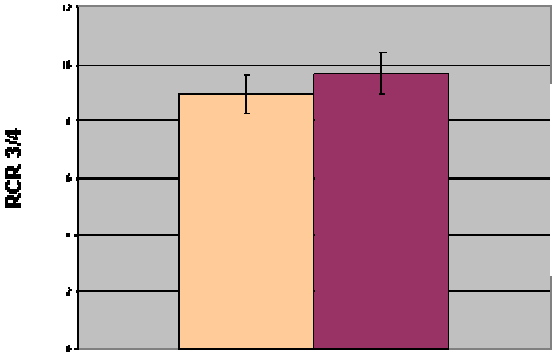
SPECIAL AND ADVANCED PREPARATIONS

EXTRACTION OF MITOCHONDRIA

Yield

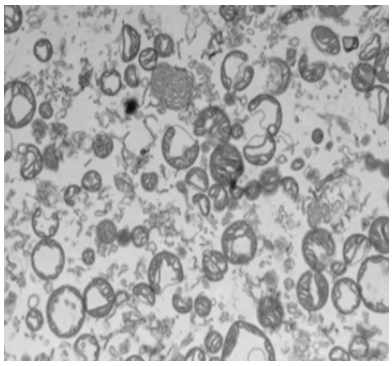


Respiration



PBI Shredder

Glass Homogenizer



Electron micrograph (4000X) of mitochondria prepared using a PBI Shredder and a PBI *MITOCHONDRIA ISOLATION KIT: RAT MUSCLE*

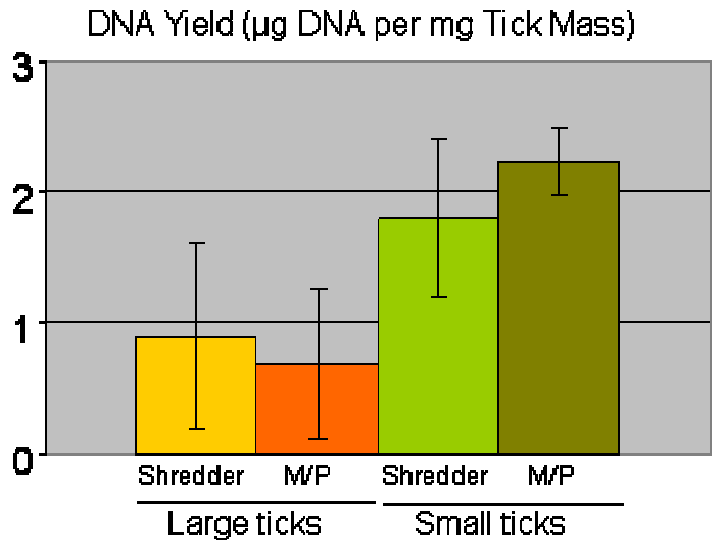
Yield and quality of mitochondria prepared from rat skeletal muscle with a PBI Shredder System compared to a Teflon/glass homogenizer are similar, but with far greater ease-of-use, lower cost and safety with the PBI system.

PBI offers specialized kits for the extraction of mitochondria from various tissues.

Mitochondria prepared from freshly harvested tissue using the PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE are intact, normal and functional, as assessed by western blotting, electron microscopy, Ca^{2+} uptake and respiration assays.

PREPARING TICKS FOR DETECTION OF TICK-BORNE PATHOGENS

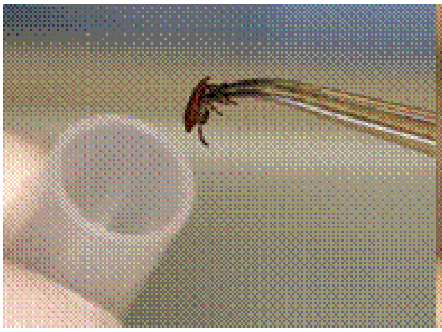
PBI Shredder vs. Mortar and Pestle



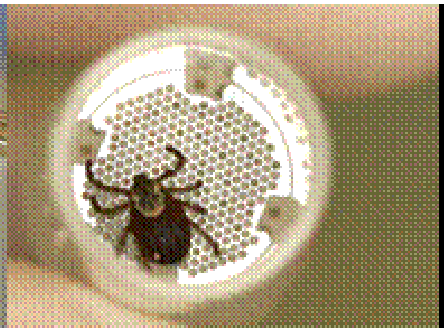
Preparation of ticks for the detection of tick-borne pathogens is often done with a razor blade and mortar/pestle. This method is both hazardous and labor intensive.

Recovering the processed tick from the mortar requires time and patience.

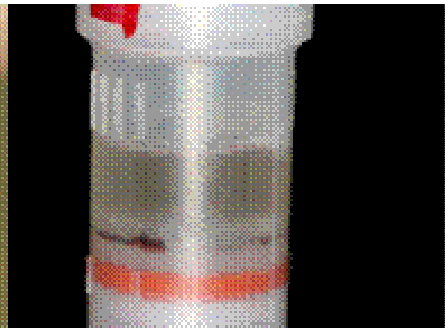
PBI Shredder Systems eliminate the labor and hazard of mortar and pestle preparation.



Placing Tick in PCT Shredder PULSE Tube (FT500-MS)



Tick in PCT Shredder PULSE Tube (FT500-MS) Prior to Shredding



Tick in PCT Shredder PULSE Tube (FT500-MS) Post-Shredding

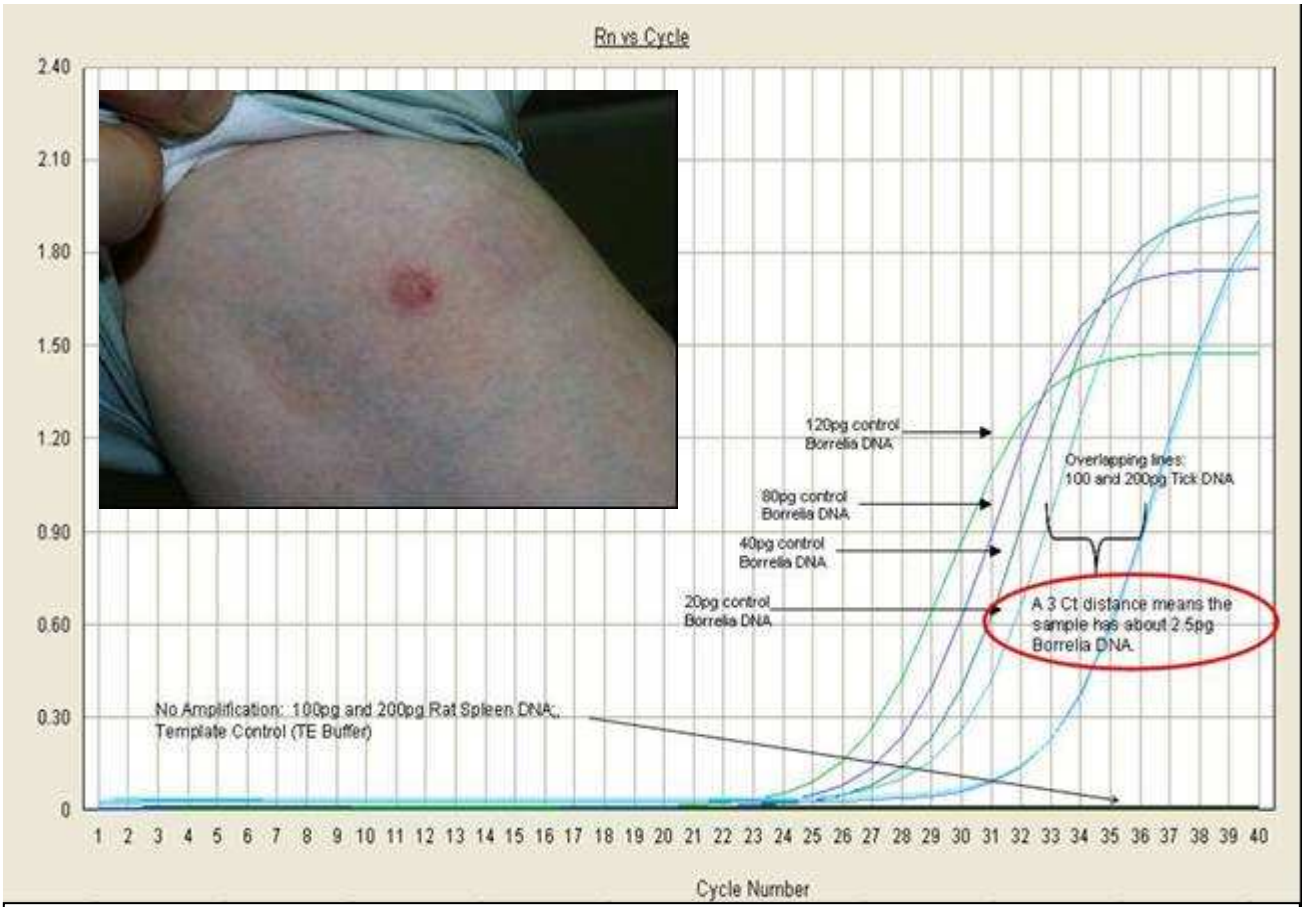
Ticks are placed in a PCT Shredder PULSE Tube affixed with a metal insert.

A Shredder Ram is added to the Tube to entrap the tick between the Ram and Metal Disk.

Buffer is then added to the Screw Cap side and the Tube is closed.

The Tube is then placed in either the *The PCT Shredder* or *The* SHREDDER SG3 for processing.

DETECTION OF THE CAUSITIVE AGENT OF LYME DISEASE (*Borrelia burgdorferi)*



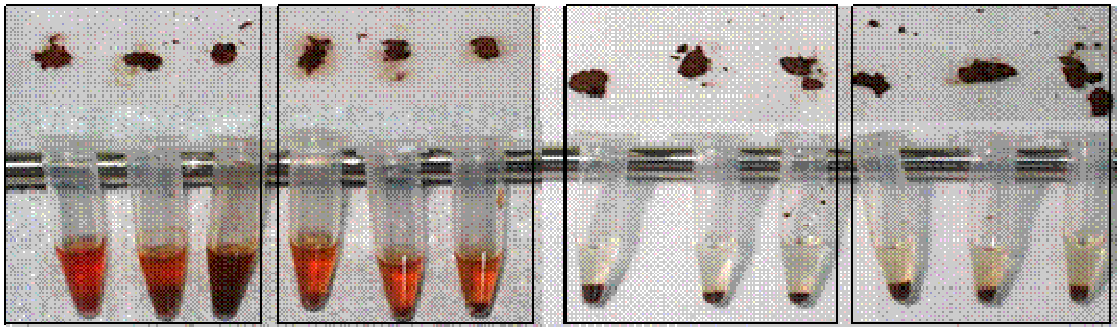
Photographic Insert: A classic presentation of a tick bite showing a characteristic "bulls eye" rash, termed Erythema migrans indicating the potential for Lyme disease.

Plot: Real-time PCR detection of *Borrelia burgdorferi,* the causative agent of Lyme disease extracted from the tick that inflicted the bite in the photograph.

Data indicate that this tick harbored *B. burgdorferi* equivalent to approximately 830,000 infectious organisms.

SHREDDING TREE BARK

Plum Tree Bark | **Pine Tree Bark**



Shredder PULSE Tube | Shredder PULSE Tube with Metal Insert | Shredder PULSE Tube | Shredder PULSE Tube with Metal Insert

Better shredding of bark is obtained using PCT Shredder PULSE Tubes (FT500-MS) or Shredder Tubes (FT500-PMS) with metal inserts.

PBI Shredder Systems are ideal for processing small samples of bark (<25mg).

The difference in the color of the extract between plum and pine bark is due to tannin content, not the effectiveness of shredding.

ADVANTAGES OF THE PBI SHREDDER SYSTEMS

Simple, Easy-To-Use

Inexpensive Hardware and Disposable Sample Containers

Compatible with Routinely Used Lab Buffers and Other Reagents

Single-Use, Closed, Versatile Processing Container

- **Self-Contained**
- **No Sample Transfer Required**
- **Reduces the Likelihood of Cross Contamination**
- **Reduces the Chance of Sample Exposure to the User**
- **Reduces the Chance of User Exposure to Potentially Hazardous Samples**
- **Excellent for Collection, Storage, Transport, and Processing**
- **Offers Full “Chain-of-Custody” Tracking from Collection to Test**
- **Available with Metal Inserts for Use with Hard Samples Such as Seeds**
- **Reduces Sample-to-Sample Variability**

Safe – No Exposed Sharp Surfaces

Portable – Excellent for Field Use

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