Introduction - What is Homogenization?

- Grinding, blending, or crushing scientific samples
- Traditionally carried out on one sample at a time

Mortar and Pestle

Dounce

Rotor-Stator

Sonicator

Pressure Cell

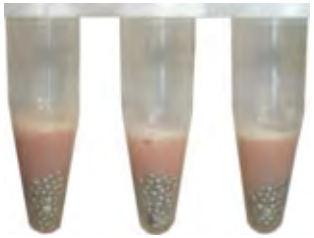
Introduction - Drawbacks to Traditional Homogenization Methods

- **Manual (Mortar and Pestle, Dounce)**
  - One sample at a time
  - Time-consuming and labor-intensive
- **Rotor-Stator Homogenizers**
  - One sample at a time
  - Operation may be messy
  - Aerosols created- problem for hazardous samples
  - Difficult to prevent heating
- **Sonicators/Ultrasonic Homogenizers**
  - One sample at a time
  - May not work without prior dissociation
  - Generates heat
- **Pressure Cell (French Press)**
  - Only for cell culture lysis
  - One sample at a time
  - Time-consuming and labor-intensive

Introduction - Bead Mill Homogenizers

- **Uses rapid agitation of beads to break down tissue and lyse cells**
- **Hands-free and automated**
- **Often high-throughput**
- **No Cross-Contamination**
  - Each sample in individual tube

Before



After

The Bullet Blender - Overview

- **High throughput benchtop homogenizer**
- **Patented striking technology**
- **Easy to use**
  - Add sample, beads and buffer to tubes
  - Place in Bullet Blender, set time and speed, press start
  - Mechanical action of beads homogenizes the sample

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The Bullet Blender - Advantages Over Other Bead Mill Homogenizers

» Samples Stay Cool

- 4°C Cooling™ in Gold models and ambient Air Cooling™ in Storm and Blue models
- Patented technology provides effective cooling at low cost
- Tubes are not encased in a holder so they are exposed to circulating air
- No “cooling cycles” necessary

» Fast and easy to load samples

- No complicated latches to fasten
- No separate holders to lose

The Bullet Blender - Advantages Over Other Bead Mill Homogenizers (cont.)

» **Risk-Free**

- 30 day money back guarantee
- 2 year warranty (extended warranties available)

» **Unparalleled Technical Support**

- Staff of experienced biologists

» **Costs 50-80% less than other high-end devices**

- Standard tubes and inexpensive beads = low cost per sample
- Blends effective technologies with efficient design
  - Low startup costs
  - Excellent performance

The Bullet Blender - How it Works

- **Patented striking technology**
  - Strikers rotate inside of the instrument and agitate the tubes
- **Requires less power than homogenizers that shake the tubes**
  - Less heat generated
  - Small footprint
  - No required cooling cycles or motor burn out
- **No separate holder for beads- quick and easy loading**

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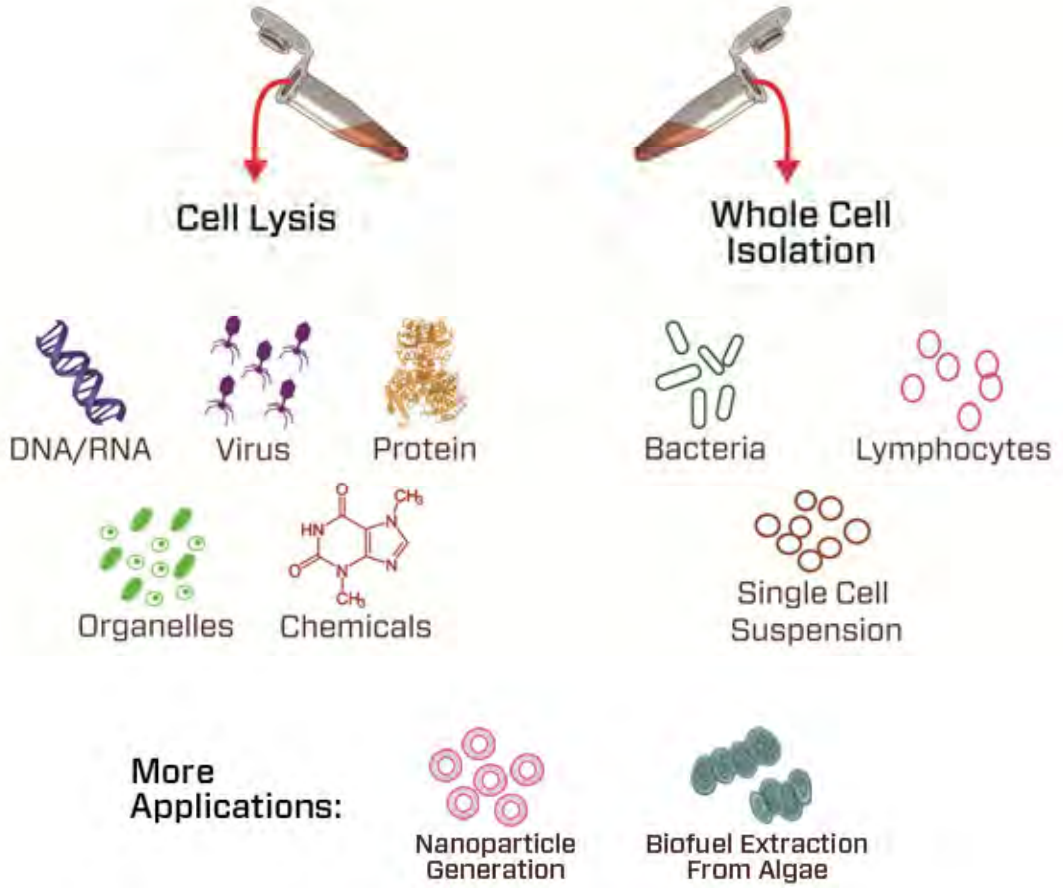
Example Results by Application - Range of Applications



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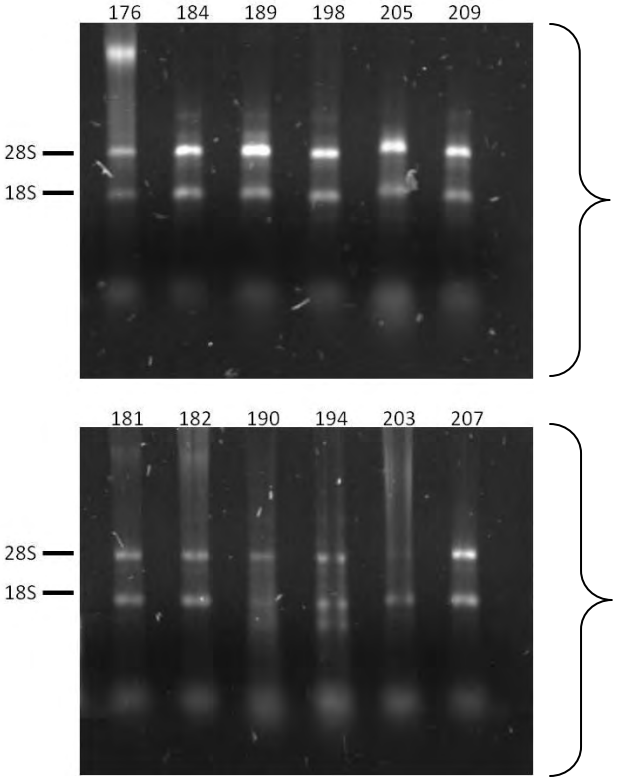
Example Results by Application - RNA Extraction



Sample #	ng/uL
176	8290,0
184	7785,0
189	10157,0
198	555,0
205	2013,0
209	1150,0
181	731,0
182	882,0
190	727,0
194	166,0
203	461,0
207	136,9

Data from melanoma cells showing the large and small ribosomal subunits (18S and 28S).
These results show that you can extract higher yields of RNA using the Bullet Blender compared to a Polytron (hand held rotor-stator homogenizer)

From **Camila Morais Melo**
ICESP- Institute of Cancer of State of São Paulo
Data was generated using mouse melanoma cellls

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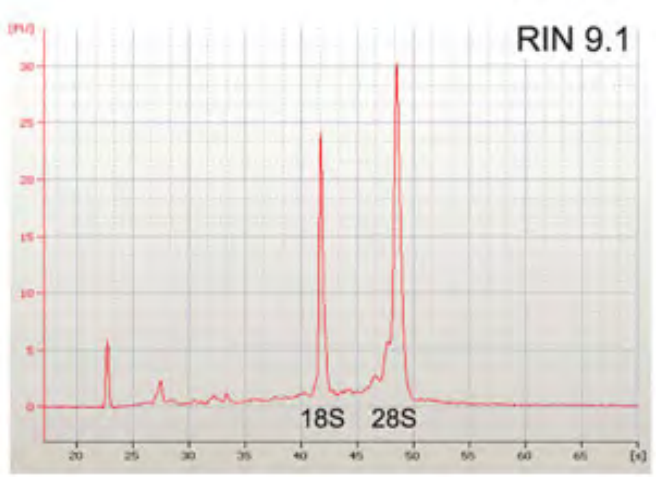
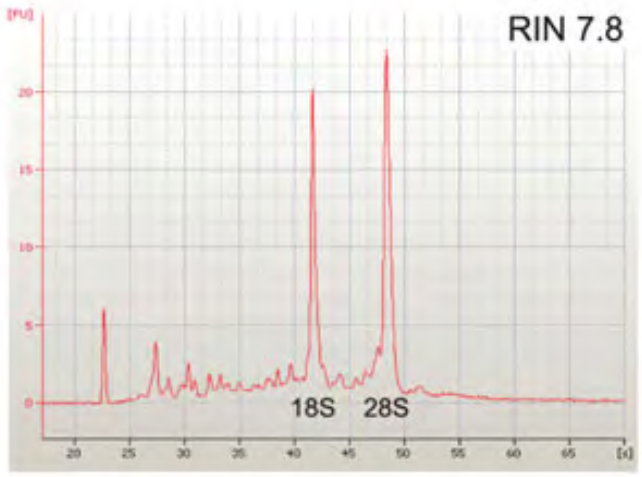
Example Results by Application - RNA Extraction cont.





RIN (RNA integrity number) of RNA extracted from mouse femur using the Bullet Blender determined with Agilent 2100 Bioanalyzer (the highest number possible is 10). These numbers are sufficient for RT qPCR, microarray and next generation sequencing applications.

From Carter LE, Kilroy G, Gimble JM, Floyd, ZE (2012) An improved method for isolation of RNA from bone. *BMC Biotechnology* 12:5

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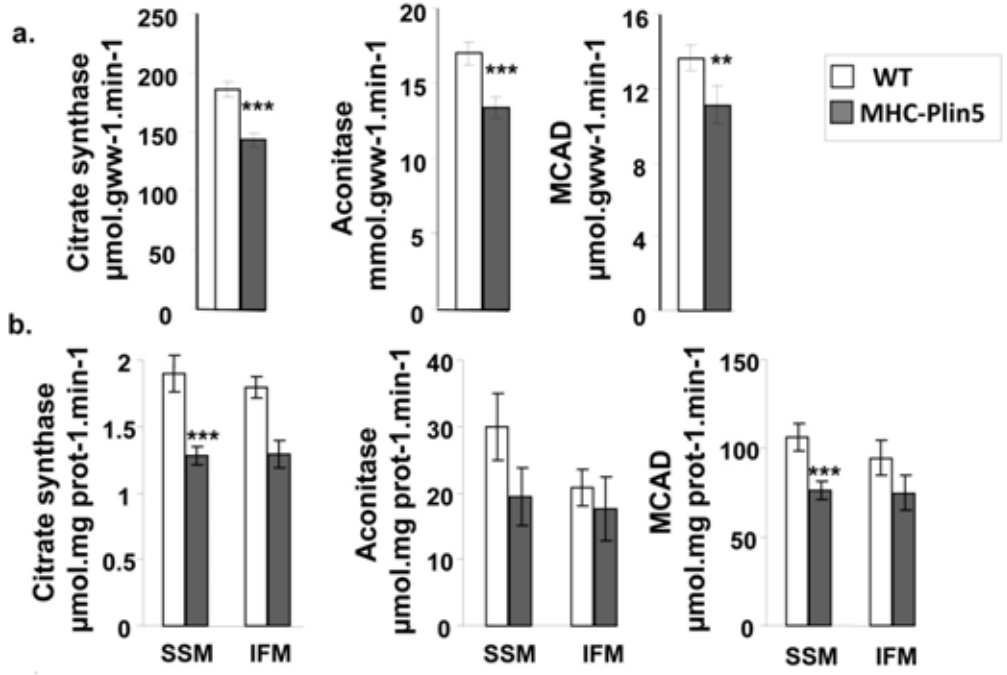
Example Results by Application - Protein Extraction



Myocardial activity of 3 oxidative enzymes extracted using the Bullet Blender.

The Bullet Blender can be used to extract fully functional native protein suitable for activity assays.

From: Wang H, Sreenivasan U, Gong DW, O'Connell KA, Dabkowski ER, Hecker PA, Ionica N, Konig M, Mahurkar A, Sun Y, Stanley WC, Sztalryd C (2013) Cardiomyocyte specific perilipin 5 over expression leads to myocardial steatosis, and modest cardiac dysfunction. *J Lipid Res* 2013 Jan 23. [Epub ahead of print]

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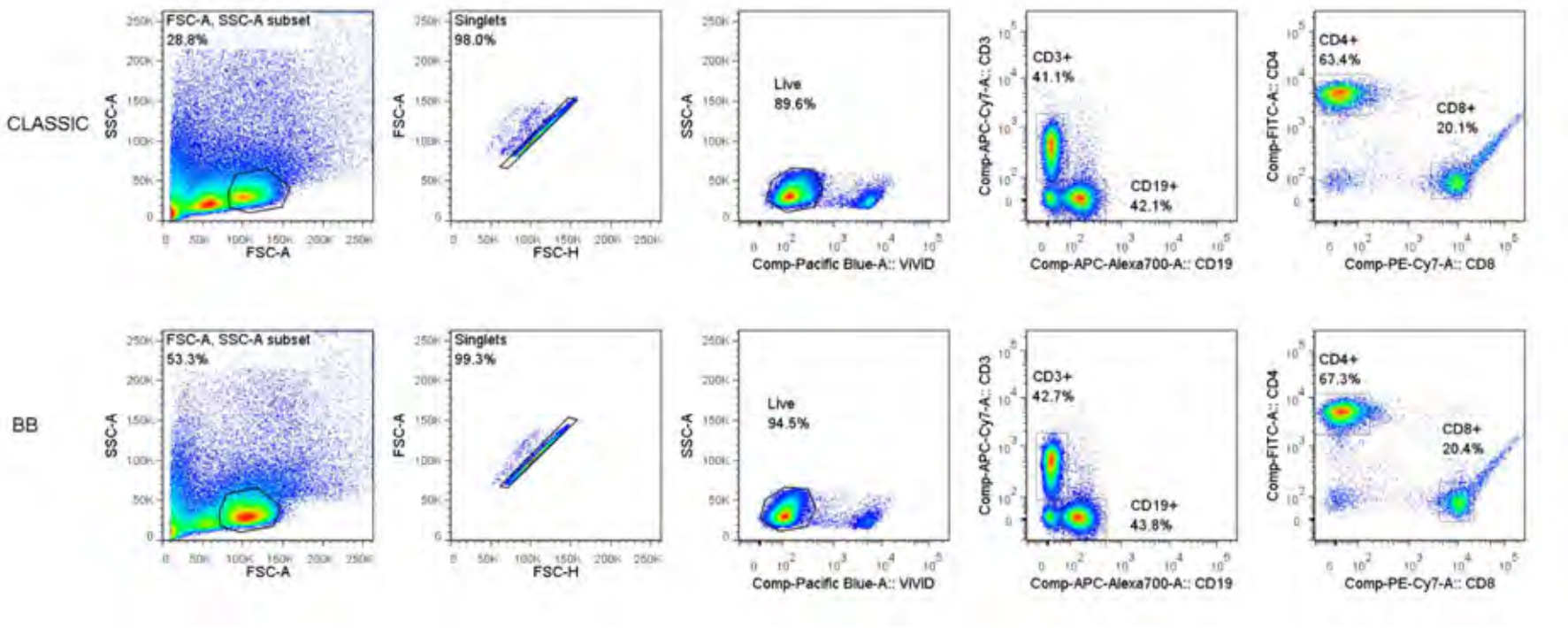
Example Results by Application - Tissue Dissociation



Fluorescence-activated cell sorting results (FACS) for spleen tissue dissociated in the Bullet Blender (BB) vs. cells isolated using enzymatic digestion (CLASSIC). Two different fluorescent labels are attached to antibodies that bind to specific types of cells, allowing them to be grouped into different “populations”. Here we see that the two methods are comparable, with good viability and yield.

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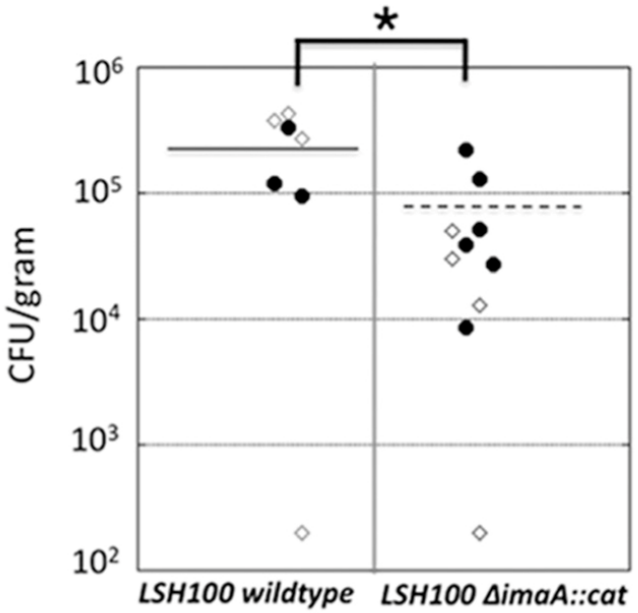
Example Results by Application - Bacterial Isolation



Isolation of *H. pylori* from stomach tissue using the Bullet Blender. Results are shown in colony forming units (CFU) per gram of tissue. Each circle represents a different mouse and the line is the mean for each group.

We see that the values between the two groups are significantly different showing that the Bullet Blender can produce precise and reproducible results for sufficient statistical analysis.

From: Sause WE, Castillo AR, Ottemann KM (2012) The *Helicobacter pylori* autotransporter ImaA (HP0289) modulates the immune response and contributes to host colonization . *Infect Immun.* 80(7):2286-96

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Example Results by Application - Chemical Analysis

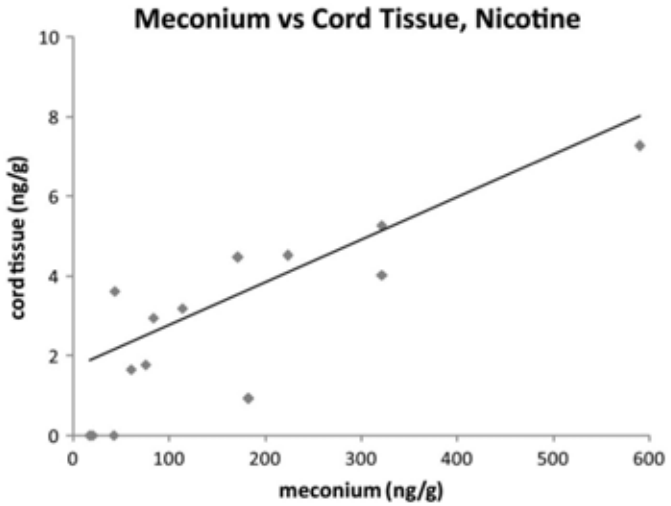

FIGURE 1. Concentration of nicotine in meconium versus cord tissue.

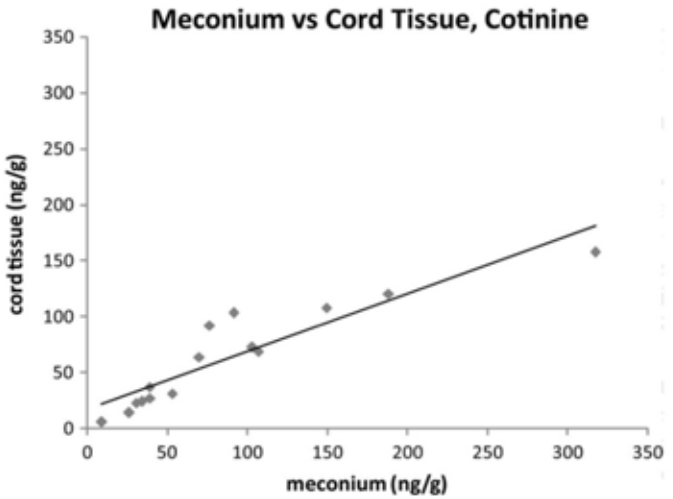

FIGURE 2. Concentration of cotinine in meconium versus cord tissue.

In this study, the Bullet Blender was used to extract nicotine and nicotine metabolites from meconium and umbilical cord tissue. The results are tight, indicating that the Bullet Blender is not introducing significant among sample variability.

From: Marin SJ, Christensen RD, Baer VL, Clark CJ, McMillin GA (2011) Nicotine and metabolites in paired umbilical cord tissue and meconium specimens. *Ther Drug Monit* 33(1):80-5

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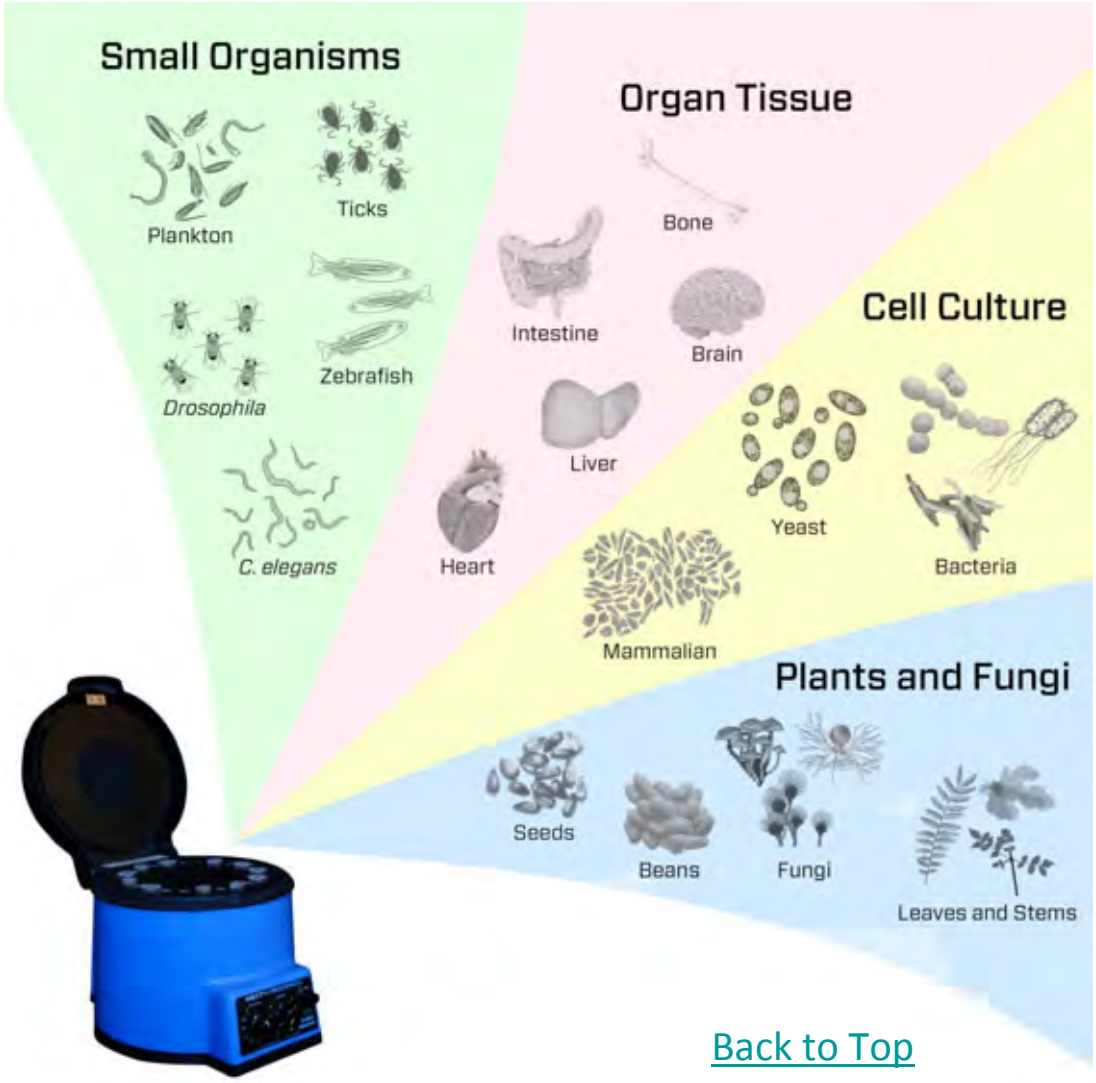
Sample Types- Range of Samples



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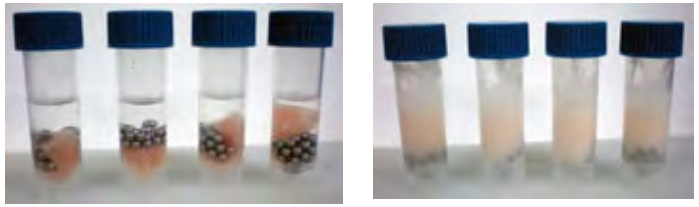
Sample Types - Organ Tissue

- Use a volume of beads equivalent to the volume of the sample
- Add a volume of liquid equal to twice the volume of beads
- Alternatively, Bead Lysis Kits can be used for most organ tissue samples
- See Bullet Blender Protocols for more specific instructions

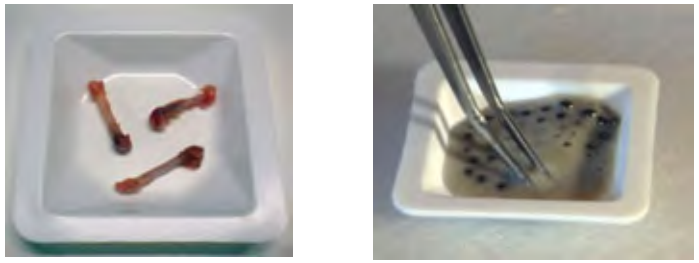
Muscle Tissue



Before After

Can also homogenize organ tissues including adipose, aorta, brain, colon, ear, eye, heart, intestine, kidney, liver, lung, lymph node, pancreas, pharynx, spleen, stomach, uterus and more.

Mouse Femur



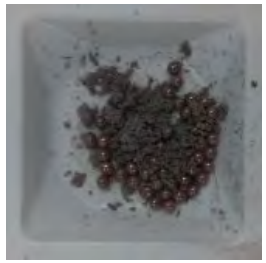
Before After

Can also homogenize tough tissues including teeth, tumor, tibia, skin, jawbone and tail snip.

Sample Types - Hair

- Add up to 50mg of hair to a 5mL Eppendorf tube
- Add 500 µL of 2.0 mm zirconium oxide beads
- No liquid added to the sample at this time
- Homogenize at speed 20 in a Bullet Blender 5E
- Reagent or buffer can be added post homogenization

Before



After

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Sample Types - Insects

- **Homogenization of insects with a hard outer shell may require a "dry homogenization" step**
  - First, process insect with beads alone (no buffer) to crush it and prevent it from floating in the liquid
  - Second, add liquid and homogenize
- **Use a 1:1:2, sample:bead:liquid volumetric ratio for best results**
- **Insect homogenization works best with stainless steel beads**

Flying Ant

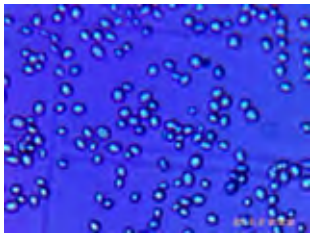
Before | After

Can also homogenize insects such as ticks, bees, spiders, flies, crickets and caterpillars.

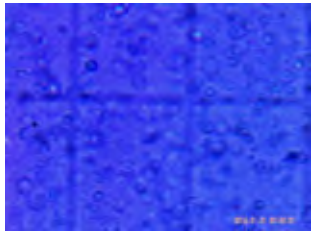
Sample Types - Cultured Cells and other Microscopic Organisms

- Use small glass or zirconium oxide beads (0.1 – 0.15 mm)
- Use a volume of beads equivalent to the volume of your pelleted cells
- Use a volume of liquid that is no more than twice the volume of your beads. More liquid than this will decrease homogenization efficiency

Yeast



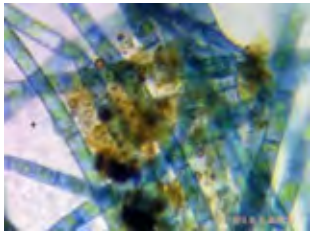
Before



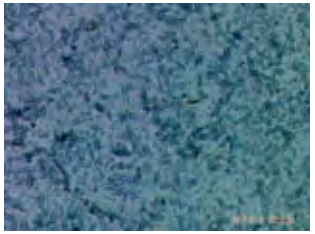
After

Can also homogenize single celled organisms such as *E. coli*, *C. albicans* and mammalian cell culture.

Algae (mixed)



Before



After

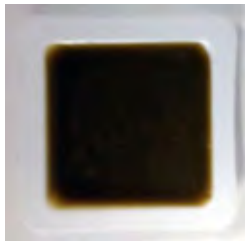
Can also homogenize microscopic organisms such as *C. elegans*, plankton and microscopic fungi.

Sample Types - Leaves, Stems and Roots

- **Slicing leaves into long, thin pieces across the main stem will make homogenization more efficient**
- **Homogenization may require a "dry homogenization" step**
  - First, homogenize only with beads- dry grinding breaks down large fibers
- **Second, add liquid and homogenize again to create a smooth paste**
- **Use a 1:1:2, sample:bead:liquid volumetric ratio for best results**

Citrus Leaves

Before



After

Can also homogenize moist plant samples such as fruit, flowers and stems.

Tomato Root

Before

After

Can also homogenize roots, tubers and rhizomes such as ginger, horseradish and potato.

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Sample Types - Dry Agricultural Samples

- Hard samples like dried corn, soybeans, shells, and grains (not all grains require “Stomping”) can be ground to a powder using the Stomper and the Bullet Blender
- First, use the Stomper to pre-crush the sample right in the tube
- Second, add beads, place in the Bullet Blender and homogenize for 5- 10 minutes
- For sensitive applications, disposable shields can be used to prevent cross-contamination
- For specific bead recommendations, please contact us at support@nextadvance.com

Choosing a Bullet Blender - Selecting the Appropriate Tube Size

» **Overloading the tube will result in poor homogenization performance**

- **24 models** are good for samples the volume of a pea or smaller (<300 µL)
- **5 models** are recommended for samples the volume of an aspirin (100 µL – 1 mL)
- **50 models** are ideal for samples the volume of a tea bag (100 µL – 3.5 mL)

Note: For wet organ tissue, 1 mL ≈ 1 g

Choosing a Bullet Blender - Microcentrifuge Models

(samples 0-300 µl)

Feature	Standard BBX24	Blue BBX24B	Storm BBY24M	Gold* BB24AU
Homogenizes up to 24 samples at a time	✓	✓	✓	✓
With Air Cooling to keep the inside of the instrument near ambient temperature		✓	✓	✓
Can homogenize samples in both screw-cap and snap-cap tubes			✓	✓
Extra-powerful homogenization for tough samples			✓	✓
With 4°C Cooling				✓

*Samples in the Gold are Air Cooled when the user chooses not to use 4°C Cooling

Choosing a Bullet Blender - Models for Larger Samples

(samples over 300 µl)





Feature	5 Storm BBY5M	5 E BBY5E	50-DX BB50-DX	5 E Gold * BB5E-AU	50 Gold* BB50-AU
Homogenization of up to 12 samples from 100 mg – 1 g (100 µl – 1 mL for plant tissue, insects and cell culture) in 5 mL screw-cap Axygen® tubes	✓				
Homogenization of up to 12 samples from 100 mg – 1 g (100 µl – 1 mL for plant tissue, insects and cell culture) in 5 mL snap-cap Eppendorf® tubes		✓		✓	
Homogenization of up to 8 samples from 100 mg – 3.5 g (100 µl – 3.5 mL for plant tissue, insects and cell culture) in 50 mL tubes			✓		✓
Extra-powerful homogenization for resilient samples (tumor, skin, bone)		✓		✓	
With Air Cooling to keep the inside of the instrument near ambient temperature	✓	✓	✓	✓	✓
With 4°C Cooling				✓	✓

*Samples in the 5E and 50 Gold are Air Cooled when the user chooses not to use 4°C Cooling

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Choosing a Bullet Blender - 4°C Cooling

- Add dry ice to the dry ice cooling compartment
- Cold air circulates past the samples, keeping them near 4°C

Choosing a Bullet Blender - Air Cooling

- Great for cold room work
- Limits heat build up during high throughput processing

Accessories - Tubes

» **RINO tubes**

- Screw-cap tubes for the Storm and Gold models
- Made of extra tough material
- Caps screw on tightly for vigorous homogenization

» **Eppendorf Safe-lock tubes**

- Recommended snap-cap tube for microcentrifuge models

» **Eppendorf 5 mL tubes**

- For Bullet Blender 5 E

» **Axygen 5 mL tubes**

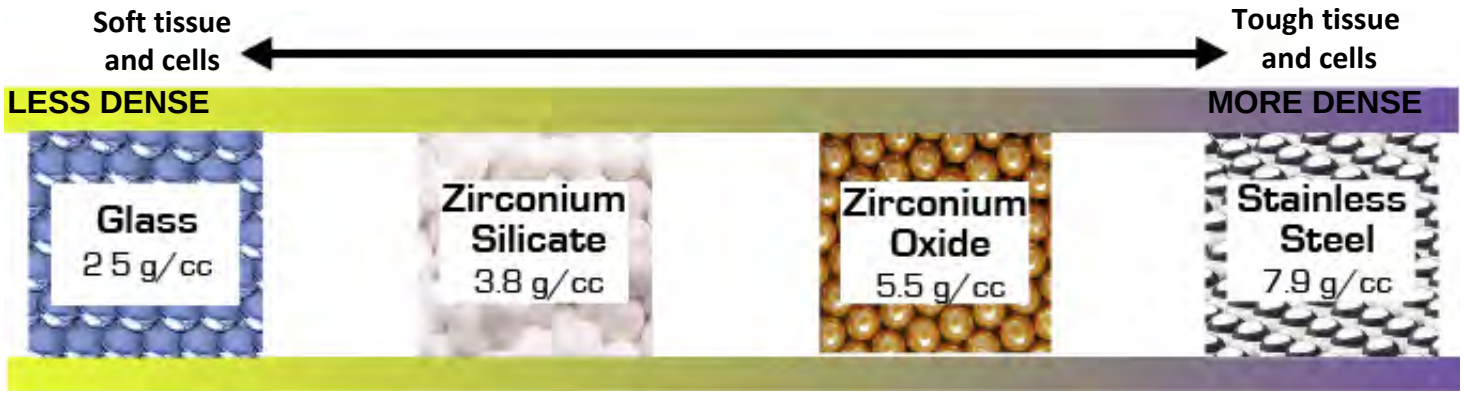
- For Bullet Blender 5 Storm

» **TPP 50 mL tubes**

- For Bullet Blender Gold 50 and Bullet Blender 50-DX

Accessories - Beads

- Beads with increasing density for tougher samples
- Smaller sizes for tiny samples and cell culture, larger sizes for tough samples and special applications
- Cone beads (UFO) feature a cutting edge for fibrous samples
- Beads sold in bulk, available RNase free
- Spoons and scoops for dispensing beads, available RNase free



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Accessories - Bead Lysis Kits

- Special bead combinations optimized for specific sample sizes and types
- Pre-dispensed into RINO™ tubes or Eppendorf® safe-lock tubes
- Achieve better results using bead combinations than with a single bead type
- Available in packs of 50 or 250, RNase free available
- See protocols to choose the kit appropriate for your sample type

Medium-Soft Organ Tissue	Tough Organ Tissue
Under 100 mg **PINK** kit	Under 100 mg **GREEN** kit
Over 100 mg **RED** kit	Over 100 mg **NAVY** kit

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Accessories - The Stomper™

- **Pre-processing tool for grinding tough, dry samples**
  - Examples: dried beans or grains, shells
- **Can accommodate 5 mL and microcentrifuge tubes**
  - No need to move samples to a different tube
- **Disposable pestle shield prevents cross-contamination**
- Click here to learn more about using The Stomper

Accessories - Magnetic Wand

- **Extremely powerful, rare earth magnet will help separate stainless steel beads from the homogenate.**
- **Use with disposable plastic wand shields to prevent cross contamination.**

Click to see a video.

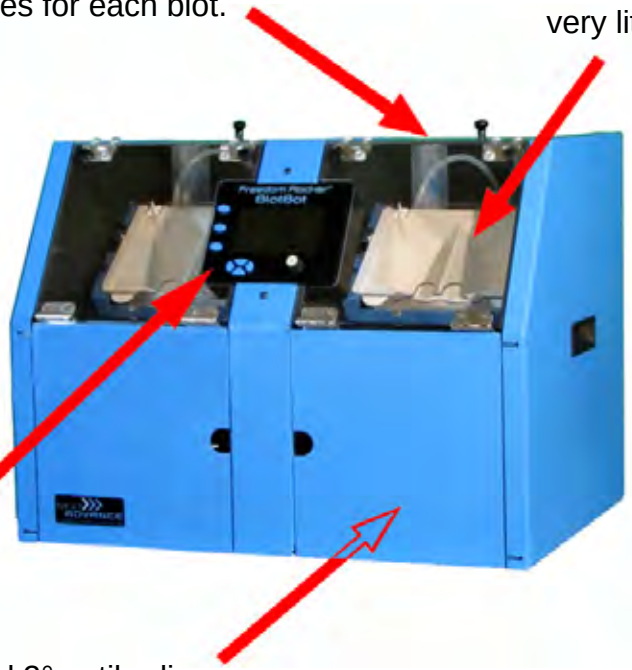
Freedom Rocker™ BlotBot®

- **Fully programmable blot/gel washer and processor.**
  - **Just add reagents and start the run.**
  - **Reagents are automatically dispensed and recovered**
  - **In a few hours, the blot will be ready for imaging.**

Use different antibodies for each blot.

Round bottom trays - use very little antibody solution

Use existing protocols.



Control panel with color graphical display – very easy to program

Recover 1° and 2° antibodies

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Freedom Rocker™ BlotBot®

- **Consistent processing every time**
  - **from day to day**
  - **from person to person.**
- **Shave months off of research project**
  - **Blots can be ready when scientist arrives at work in the morning. The next sequential test can start the same day.**
- **Use very little antibody solution per blot**
  - **Use different antibodies for each blot**
  - **Recover primary and secondary antibodies**

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Freedom Rocker™ BlotBot® Models

BlotBot 120

BlotBot 240

Simultaneous processing of:

- **1 midi or**
- **2 mini size blots**

- **2 midi or**
- **4 mini size blots**

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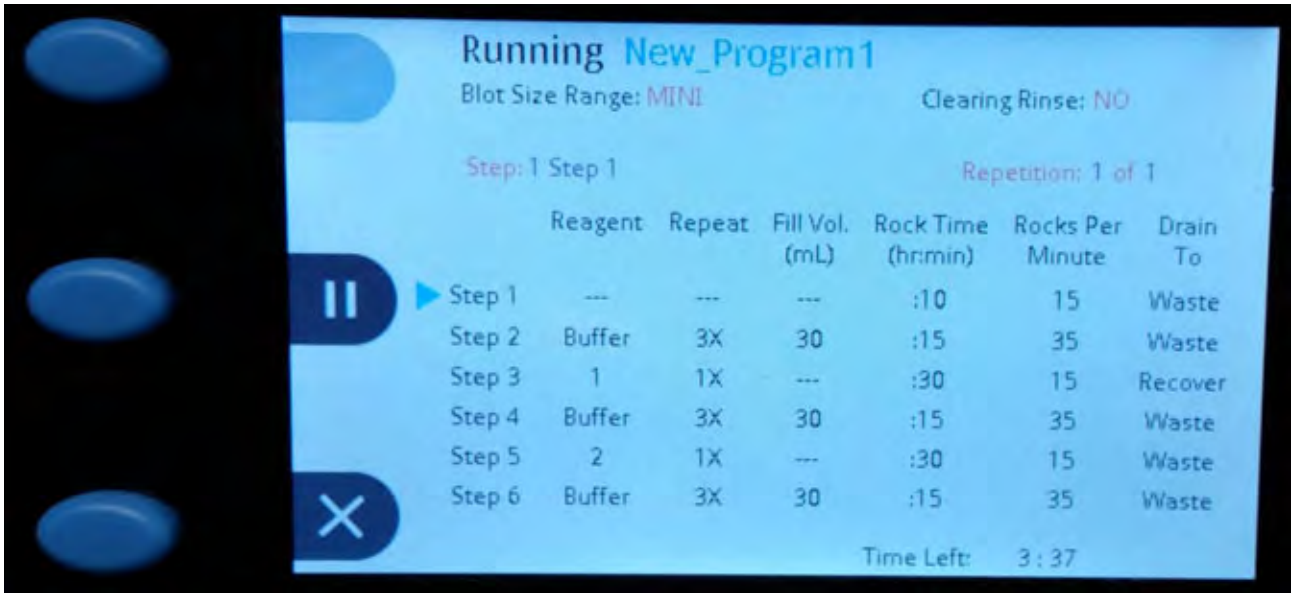
Freedom Rocker™ BlotBot® Programming



- Save 8 protocols.
- Highly customizable – run up to 9 steps, each step select: a reagent or buffer; volume; rock duration; rock speed; and whether to recover reagent.
- Simple to use programming interface.

Other Products - Pressure Injection Cells

- Pack capillary columns for LC/MS
- Load samples into mass spectrometers
- Save money
- Get accurate results
- Integrated stir plate available

Other Products - Pressure Injection Cell Models

PC1000
Transparent (acrylic) body with
1000 psi maximum pressure

PC77
2500 psi maximum pressure

PC8500
8500 psi maximum pressure

PC77-MAG
All pressure cells available with
integrated magnetic stir plate

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Other Products - Infinity Rockers

- **4 year warranty**
- **Digital speed control (0.1 – 80 cycles/min)**
- **Adjustable tilt**
- **3 platform sizes available**
- **Multi-platform models and stacking trays available**
- **Available with custom rocking programs**
  - Pause cycles and asymmetric rocking
  - For specialized mixing applications such as viral transduction, plasmid transfection, hybridization and microfluidic applications

Other Products - Next Rockers

- Value laboratory rocker
- Quiet operation
- Adjustable tilt
- 2 platform sizes available
- Stacking trays available for increased capacity

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