Bead Beating: A Primer

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INTRODUCTION

Bead beating is an effective mechanical method used to disrupt a wide range of biological samples. At a minimum, bead beating is accomplished by rapidly agitating a sample with grinding media (beads or balls) in a bead beater (device that shakes the homogenization vessel). Bead beaters have been designed to homogenize samples in microwell plates, tubes, or vials with beads or balls that are made of glass (silica), ceramic (zirconium) or steel. Samples can be processed with or without buffer or solvent at either ambient or cryogenic temperatures.

Researchers often bead beat because it is a very efficient method to break down most samples. Unlike handheld rotor-stators, bead beaters do not directly contact the sample, thereby reducing cross-contamination when multiple samples are processed. Bead beating instruments vary in the number of samples that can be processed at a time, physical motion, and the speed at which the samples are agitated.

Types of Bead Beaters

A variety of devices are used to bead beat samples. In all cases, the tube, vial or plate is shaken so that grinding media can impact and disrupt the sample. Vortexers, the simplest (and least effective) bead beater, work by swirling the sample and beads/ balls in a motion that promotes disruption of microorganisms. Other bead beaters, including dental amalgamators and shaking mills, oscillate tubes, often in a figure-eight motion, which allows for crushing and grinding of samples. High throughput homogenizers, which can process samples in deep well plates in addition to other formats, have a linear motion that focuses the kinetic energy of the grinding media on the sample instead of on the sides of the container. Extremely resilient samples, such as seeds, are most effectively homogenized via linear motion bead beaters. While vortexers are limited to processing microorganisms, other beat beaters have a wider range of sample processing capabilities (Table 1).

Single and multitube vortexers can be used to disrupt microorganisms, but are very ineffective at homogenizing solid tissues. Vortexers are a good choice when a limited number of samples require processing, or for laboratories on very tight budgets. Adding beads and a suspension of microorganisms to a snap cap tube and holding it on a vortex mixer for a minute will result in disrupted cells. However, this method is generally less than half the effectiveness of dedicated bead beaters. Some vortexers have been modified to function more like a bead beater. For instance, the Pulsing Vortex Mixer can process 12 samples in microcentrifuge tubes at a fraction of the cost of a dedicated bead beater. Though more expensive, a Multitube Vortexer can accommodate full racks of tubes or microwell plates, thus allowing higher throughput processing.

Table 1. Homogenizer Characteristics and Capacities.

	Vortexer	HT Mini™	HT 24™	HT Homogenizer™	1600 Mini G™	2010 GenoGrinder®
Characteristics						
Motion	Vortex	Arch	Figure 8	Linear	Linear	Linear
Speed - High	3000 rpm	4000 rpm	4200 rpm	1600 rpm	1500 rpm	1750 rpm
Speed - Low	Varies	2800 rpm	2400 rpm	50 rpm	500 rpm	500 rpm
Timer	Available	0-60 sec.	0-90 sec[1]	0-10 min.	0-10 min.	0-20 min.
Pulsing Feature	Available	No	Yes	No	No	No
Cryogenic Grinding	No	No	No	No	Yes	Yes
Grinding Formats						
Microcentrifuge Tubes	12	-	-	24	50	100
Disruption Tube (nonskirted)	-	3	24	24	50	100
Disruption Tube (skirted)	-	-	24	24	50	100
Deep Well Plate	-	-	-	1	2	4-6[2]
4 ml Grinding Vials	-	-	-	24	48	96
15 ml Grinding Vials	-	-	-	5	10	20
15 ml Conical Tubes	-	-	-	-	12	24
50 ml Conical Tubes	-	-	-	-	6	12

[1] The HT 24 can run for 90 seconds continuously, but can cycle 10 times using the pulsing feature. When this feature is active, a pause (1-120 seconds) is inserted between the run times. Thus, total processing time can be up to 15 minutes with pauses of as little as 1 second.

[2] Initially, the GenoGrinder 2010 could be adapted to hold four deep well plates. The clamp assembly was later modified to hold up to six plates.

Multitube bead beaters like the HT Mini™ and the HT 24™ use a high speed oscillating motion to homogenize microorganisms and tissues samples. The HT Mini™, which is based on the design of a dental amalgamator, can effectively homogenize microorganisms and small tissue samples by rapidly shaking microcentrifuge tubes clamped in a small arm back and forth between 2800 and 4000 rpm. Microorganisms placed in tubes with grinding beads can be homogenized in as little as 30 seconds. The HT Mini™ has been designed to hold one to three 2 ml screw cap nonskirted disruption tubes. The HT 24™ can oscillate up to 24 samples in 0.5 ml or 2 ml disruption tubes in a figure-eight multidirectional motion at speeds between 2400 to 4200 rpm. Heat, which can be generated as a result of the high-speed agitation, can affect the integrity of many biomolecules. Consequently, the HT 24™ has a pulsing feature that can be programmed to operate in short bursts with interval rest periods, allowing heat to dissipate between cycles.

High throughput homogenizers are specifically designed for processing samples in deep well plates and vials, but they can also accommodate microcentrifuge tubes and small bottles, allowing for the processing of large samples. Organs that are too large to be processed in a dental amalgamator or multitube bead beaters can be homogenized in high throughput homogenizers. Although these instruments can be expensive, they are capable of efficiently processing hundreds to thousands of samples daily. Models include the HT Homogenizer™, 1600 Mini G™, and 2010 GenoGrinder®. These homogenizers have a range of capabilities, which is reflected in their cost.

All high throughput homogenizers can process samples in plates or vial sets in SBS (Society for Biomolecular Science) formats (e.g., 24 and 96 well). This format permits liquid handling robots to process samples downstream of homogenization. The HT Homogenizer™ can process a single deep well plate, a single rack of disruption tubes, up to twenty four (24) 4 ml vials, or five 15 ml vials at a time. The 1600 Mini G™ has twice the processing capability of the HT Homogenizer™, as well as the ability to process samples in tall centrifuge tubes (15 and 50 ml conical tubes) and cryogenically in a Cryoblock. The 2010 GenoGrinder® has all of the advanced features as the 1600 Mini G™ (i.e., cryogenic grinding, digital controls, and sleek design), but with much greater capacity. It can process up to six deep well plates, four vial sets, and multiple racks of disruption tubes at a time.

Sample Considerations

The term sample resiliency refers to the degree by which a sample is unscathed following bead beating. Some samples, such as thymus and liver, have very low resiliency and are relatively easy to homogenize by bead beating. Other samples, such as skin, fish scale, and nail, are very resilient and are homogenized with great difficulty. A few samples are totally resilient, like palm nuts, which remain virtually untouched when using even the largest, most dense grinding balls. Luckily, most samples are of moderate resiliency and can be effectively homogenized.

The key to effectively homogenizing samples is to find a balance between sample mass, vessel volume and grinding media size. With very soft samples, like liver, several hundred milligrams can be processed in a 2 ml disruption tube with a 5/32" grinding ball in a HT Mini™ or HT 24™. However, a 20 mg sample of rat skin, which is very durable, may require a 4 ml vial and a 3/8" grinding ball under cryogenic conditions using a GenoGrinder®. This highlights an important point: it is easy to purchase different grinding vessels and media, but much more difficult to switch homogenizers. While many laboratories have bead beaters that have been inherited, it is very important for researchers purchasing new homogenizers to consider the potential sample types that will be processed in the future (Table 2).

Table 2. Cross reference of sample type and bead beater capability.

		Bead Beater Model				
Sample	**Vortexer**	**HT Mini™**	**HT 24™**	**HT Homogenizer™**	**1600 Mini G™**	**2010 GenoGrinder®**
Microorganisms						
Bacteria	+	++	++	++	++	++
Yeast	+	++	++	++	++	++
Fungi	+	++	++	++	++	++
Plant						
Pollen		++	++	++	++	++
Leaf Tissue		++	++	++	++	++
Stem Tissue		++	++	++	++	++
Callus		+	+	++	++	++
Seeds				++	++	++
Root		+	+	++	++	++
Bark				++	++	++
Animal						
Soft Animal Tissue		++	++	++	++	++
Fibrous Animal Tissue		++	++	++	++	++
Elastic Animal Tissue		+	+	+	+	+
Hard Animal Tissue				+	+	+

Homogenization Parameters

In order to thoroughly homogenize a sample, the nature of the sample and its mass (size) must be matched with both a suitable vessel (vial, plate, tube) and the appropriate grinding media. Bead beating can be done in a variety of vessels, including disruption (microcentrifuge) tubes, grinding vials and microwell plates. Even square 125 ml plastic serum bottles can be used for very large samples on any one of the high throughput homogenizers. Whatever the format, sample vessels must be able to withstand the impact of grinding beads and balls. This ability is determined by the mass of the grinding beads/balls and the type of plastic used to make the vessel. Polycarbonate is the best plastic for bead beating because it is durable, clear and impact resistant, even at cryogenic temperatures. Due to the fact that many organic solvents are incompatible with polycarbonate, durable polyethylene can be used instead. Polypropylene disruption tubes and deep well plates work well with small beads but will often crack when used with metal grinding balls.

The size (mass and volume) and type of sample must be matched with a homogenization vessel of an appropriate volume as well as grinding media that can effectively homogenize the sample. Grinding media must be able to move freely to impact the sample; therefore, homogenization vessels must not be overfilled with sample, beads and/or buffer. Generally, the samples should take up no more than a sixth of the volume of the vessel, and grinding media should take up no more than a third of the volume of the vessel. As well as overfilling vessels, processing very small samples in larger grinding vials can also lead to inefficient homogenization. In such instances, it may be necessary to process the samples for a longer period of time.

Grinding media used during bead beating can be divided into beads, grinding balls, ceramic satellites and/or grinding resins. Grinding beads are a pool of beads that fall within a specific size range, while grinding balls are spherical and precision ground with a specific diameter. Beads with smaller diameters are best for disrupting microorganisms like bacteria and yeast, while larger beads or grinding balls are best for homogenizing animal and plant samples. Metal balls are used to grind resilient and fibrous samples. Because stainless steel balls may oxidize in the presence of phenolic compounds and interfere with subsequent processing, cylindrical or Saturn-shaped ceramic satellites are a useful alternative. Grinding resins are sharp, irregular shaped composites, such as garnet, that can also be effective at shredding resilient or fibrous samples.

The inherent properties and composition of grinding media affect sample homogenization (Table 3). Zirconium, silica and stainless steel (an alloy containing iron, carbon and chromium) are widely used as grinding media. Higher density grinding media (i.e. stainless steel and zirconium compounds) are generally more effective than silica at homogenization. Stainless steel and zirconium media, however, generate significant heat during processing. Stainless steel can also oxidize in extraction buffers containing phenol, which can potentially interfere with downstream applications. Additionally, silicate and chromium sheared from beads during bead beating can inhibit some enzyme reactions. Hence, no one media is good for all applications.

Based on the size of the grinding media and composition of the homogenization vessel, not all permutations of media and vessels are compatible (Table 4). Generally, small beads can be used with any vessel, but the same cannot be said for larger grinding balls. Polypropylene deep well plates are sometimes manufactured with very thin walls, which can crack when impacted by grinding balls. Stainless steel 5/32” balls are routinely used with plates, but in many circumstances can punch through the bottom of the wells. Also, larger balls are physically too wide for many plates and disruption tubes.

Table 3. Properties of common grinding media.

	Silica	Zirconium Silicate	Zirconium Oxide (Yttria Stabilized)	Zirconium Oxide (Ceria stabilized)	Stainless Steel
Density (g/cc)	2.25	3.84	6.0	6.20	7.9
Durability	Low	Medium	High	High	Medium
Relative Hardness	+	++	++++	++++	++

Table 4. Grinding media compatibility with tubes, plates, and vials.

	Homogenization Vessel				
Grinding Media Size	Disruption Tubes	Deep Well Plates	Polyethylene Vials (4 ml)	Polycarbonate Vials (4 ml)	Polycarbonate Vials (15 ml)
100 µm – 3.0 mm Ceramic Beads	+	+	+	+	+
2.8 mm Metal Balls	+	+	+	+	+
5/32” Metal or Ceramic Balls	+	+/-	+	+	+
6 mm Ceramic Satellites	+	+/-	+	+	+
5/16” Metal Balls			+	+	+
3/8” Metal Balls			+	+	+
7/16” Metal Balls					+

In general, grinding beads, balls, or satellites (non-spherical precision ceramic balls) should be cleaned prior to use to remove contaminants and inhibitors because some grinding media can be flush with impurities[3]. Acid washing beads is the most common method to remove contaminants. Additionally, heat treating beads eliminates them as a potential source of nuclease or nucleic acid contamination. Grinding balls must be degreased prior to use by repeatedly washing in organic solvents.

When extremely small tissue samples or a limited number of cells are disrupted, there is a chance that the desired analyte (e.g., specific DNA sequence or protein species) may be lost due to non-specific adsorption to the beads. Small glass and zirconium beads (100 to 800 micron) have a relatively large surface area that can bind analytes, effectively lowering detection limits on assays. Thus, low binding beads were developed to reduce non-specific binding. The beads allow more analyte to remain in solution and have been shown to aid in increasing assay detection limits.

Cryogenic Homogenization

The labile and/or resilient nature of some samples requires that they be pulverized cryogenically. Traditional methods use a mortar and pestle chilled with liquid nitrogen for cryogenic grinding of larger samples, but this is impractical for high-throughput processing of small samples. High throughput homogenizers, such as the Mini G™ and GenoGrinder®, are capable of processing samples cryogenically using polycarbonate vials and grinding balls. While many other plastics can become very brittle, polycarbonate can withstand cryogenic temperatures. The 4 ml and 15 ml polycarbonate vials are designed with reinforced bottoms so that they will withstand the impact of metal grinding balls. Polypropylene plates, tubes, and vials can crack if used for cryogenic grinding.

Cryogenic grinding is a relatively straight forward process. A vial containing the sample and grinding ball is chilled with liquid nitrogen by placing it in a bath prior to homogenization. The vial should be chilled without a cap, as caps are typically polypropylene and will crack if chilled. Using this simple method, chilled vials are removed from the liquid nitrogen bath, rapidly capped, racked, and placed in a bead beater for processing. While this is a useful homogenization method when working with resilient samples that are made brittle by the cold, it is not ideal for samples that contain heat labile molecules because samples will warm during processing. To offset generated heat, vials can be placed in a liquid nitrogen chilled Cryoblock (a machined aluminum block) to keep the vials and sample at cryogenic temperatures throughout processing. Cryoblocks can only be used with the 1600 Mini G™ and 2010 GenoGrinder®, as these two homogenizers are designed to carry the additional weight. The 1600 Mini G™ can accommodate one Cryoblock while the 2010 GenoGrinder® can hold two Cryoblocks.

For more details on cryogenic homogenization, see Appendix B.

MICROORGANISMS

Bacterial Homogenization

Small size and cell concentration/density are important parameters to consider when bead beating bacteria. Whether disrupting bacterial cultures directly or following concentration by centrifugation, bead beating of bacteria is most effective with 100 µm zirconium or silica beads. With dilute samples, low binding beads will reduce the loss of analytes during processing. Depending upon the beat beater, samples can be processed in 0.5 ml or 2 ml disruption tubes, deep well plates, 4 ml polycarbonate or polyethylene vials, and 15 ml polycarbonate vials. Larger volumes or samples with high concentrations of cells typically require longer processing times. Likewise, linear homogenizers require longer processing times than the HT Mini™ or HT 24™.

Procedure

1. Both dilute and concentrated bacteria can be homogenized effectively. Pellet bacterial cells by centrifugation and resuspend in a suitable homogenization buffer. It is important to limit the size of a pellet that is resuspended for bead beating. Samples that are too thick are difficult to disrupt since the small beads must not only crack cells, but also fight against the viscosity of the suspension. Generally, the pellet from 2 ml of cultured broth can be disrupted in 2 ml tubes or plates, while the pellet from up to 15 ml of culture broth can be disrupted in 4 ml vials; a pellet from up to 100 ml culture broth can be disrupted in 15 ml vials.
2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	**Deep Well Plates (square wells)**	**4 ml Vials**	**15 ml Vials**
Buffer Volume	600 µl	600 µl	1.5 ml	6 ml
Bead Volume (100 micron beads)	400 µl	300 µl	0.8 ml	3 ml
Processing Time	2-5 minutes	5 minutes	5 minutes	5-10 minutes
Bead Beater Speed	High	High	High	High

[3] All grinding media manufactured by OPS Diagnostics is cleaned, treated, and ready-to-use.

3. Process the samples as stated in the table. For heat sensitive samples, the processing can be divided into short bursts followed by a cooling period (known as pulsing).
4. Sample homogenization cannot be monitored visually because the cells are too small. Disruption can be checked using a marker enzyme, such as lactate dehydrogenase, which is liberated from cells during processing.
5. Proceed to purification and/or analysis when cells are sufficiently disrupted.

Suggested Products

Beads:	100 µm Zirconium (BAWZ 100-250-15)
Pre-filled Tubes:	100 µm Zirconium (PFAW 100-100-02)
Homogenization Vessel:	See Appendix A
Homogenizers:	See Appendix A

Environmental Sample Homogenization

Air, water, soil, sediments, and surfaces supporting biofilm can loosely be grouped as environmental samples. A common characteristic of environmental samples is the presence of both biotic and abiotic components, with the former representing a diverse collection or community of microbes, animals and plants (to various degrees). Depending upon the density of the biotic component, environmental samples are often concentrated by centrifugation or filtration prior to homogenization. The biotic component of air and water can be concentrated by membrane filtration. The filter and retained materials are then subsequently homogenized by bead beating. Water can also be centrifuged to pellet microorganisms, though this can be impractical with larger volumes. Biofilms grown on the inside surface of glass tubes can be disrupted by placing the tube in a vial with a bead mixture. The homogenization of soil and sediments must not only disrupt the biological component, but also disaggregate soil particles that may house microorganisms. Soil and sediments can be processed directly with mixed beads and an extraction buffer.

In all cases, environmental samples usually require a mixture of different beads and balls to effectively disrupt the range of organisms and particles present. Samples with significant amounts of abiotic matrices can be homogenized with a combination of one or more large beads to facilitate general breakage. A mixture may include a couple of large balls (e.g., 4 mm) to break up soil particles, beads ranging in size from 800 µm – 1.4 mm to facilitate fungi breakage and 100 µm beads to disrupt bacteria. Depending upon the beat beater, samples can be processed in disruption tubes, deep well plates, 4 ml polycarbonate vials, and 15 ml polycarbonate vials.

Procedure

1. A mixture containing small (100 µm), medium (400-800 µm), and large (1.4 mm to 4 mm) beads is recommended for environmental samples. Typically, the mixture contains many small beads and only one or two large beads. Following are recommended bead combinations for homogenization in 2 ml disruption tubes:

Matrix	Sample	Bead Mixture
Membrane Filter	¼ section of 47 mm membrane filter in 2 ml disruption tube	100 mg of each 100 µm and 400 µm silica beads
Soil/Sediment	250 mg in 2 ml disruption tube	100 mg each of 100 µm silica and 500 mg of 400 µm beads and two 4 mm glass beads
Biofilm	Stationary phase with biofilm which fits in a tube or vial (objective is to remove the film from the support).	1/6 volume of 1.0 mm zirconium beads. Subsequent processing may require smaller beads.

2. Prepare samples as described above and add extraction buffer. The recipe and volume of the buffer will vary based on subsequent processing steps, but, in general, the volume should be kept between 0.5 and 1.0 ml. For larger vials, the amount of sample and beads can be scaled up accordingly.
3. Process samples on high speed for 2 minutes. For heat labile samples, shorter processing bursts can be alternated with cooling periods, e.g., process for 15 seconds then cool for 2 minutes.
4. The slower high throughput machines may need longer processing times than the HT Mini™ and HT 24™.

Suggested Products

Beads:	100 µm Zirconium (BAWZ 100-250-15), 100 µm Silica (BAWG 100-200-10), 400 µm Silica (BAWG 400-200-04), 1.0 mm Zirconium Beads (BAWZ-1000-250-33), 4 mm Glass Beads (BAWG 4000-200-18)
Pre-filled Tubes:	Mixed 100 µm, 1.4 mm Zirconium & 4 mm Silica (PFMM 4000-100-28)
Homogenization Vessel:	See Appendix A
Homogenizers:	See Appendix A

Yeast Homogenization

Yeast is a vegetative single cell fungus that can be effectively cracked open via bead beating. Small yeast cells, such as *Pichia*, are best homogenized with 200 µm zirconium beads. Larger yeast cells, such as *Saccharomyces,* are best disrupted with 400 µm silica or zirconium beads. Yeast samples with low cell numbers can be homogenized with low binding beads, which lose fewer analytes to non-specific adsorption. Depending upon the bead beater, samples can be processed in 2 ml disruption tubes, deep well plates, 4 ml polycarbonate or polyethylene vials, and 15 ml polycarbonate vials.

Procedures

1. Both dilute and concentrated yeast can be homogenized effectively. Pellet yeast cells by centrifugation and resuspend in a suitable homogenization buffer. It is important to limit the size of a pellet that is resuspended for bead beating. Resuspended yeast pellets can be very viscous, which will work against efficient disruption. Generally, the pellet from 2 ml of cultured broth can be disrupted in 2 ml tubes or plates, cells from up to 15 ml culture broth in 4 ml vials, and a pellet from up to 100 ml culture broth in 15 ml vials.
2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	**Deep Well Plates (square wells)**	**4 ml Vials**	**15 ml Vials**
Buffer Volume	600 µl	600 µl	1.5 ml	6 ml
Bead Volume (400 micron beads)	400 µl	300 µl	0.8 ml	3 ml
Processing Time	5 minutes	5 minutes	5-10 minutes	5-10 minutes
Bead Beater Speed	High	High	High	High

3. Process the samples as stated in the table. For heat sensitive samples, the processing can be divided into short bursts followed by a cooling period.
4. Disruption of yeast can be monitored microscopically. Using phase contrast, intact yeast cells appear refractile (bright) as the cells act like a small lens. Disrupted yeast cells look black or gray, which are often termed “ghosts”. Visually inspect the yeast cells (dilution may be needed) and count or estimate the percentage of ghost cells in the population. Typically, bead beating should yield 90% or greater ghost cells.
5. Continue cell processing if sufficient lysis has not occurred.

Suggested Products

Beads:	400 µm Zirconium (BAWZ 400-250-30) for Saccharomyces; 200 µm Zirconium (BAWZ 200-250-07) for Pichia
Pre-filled Tubes:	400 µm Zirconium (PFAW 400-100-30) for Saccharomyces; 200 µm Zirconium (PFAW 200-100-03) for Pichia
Homogenization Vessel:	See Appendix A
Homogenizers:	See Appendix A

Fungal Homogenization

Filamentous fungi, pseudomycelia and fungal bodies can be homogenized with a variety of beads. Hyphae are relatively easy to shear, while dense mycelia and fruiting bodies (e.g., mushrooms, bracket fungi) can be much more difficult. Loosely packed mycelia are effectively disrupted with 800 µm zirconium or silica beads. However, as the density of the mycelium increases, as with fruiting bodies, larger beads and small grinding balls are needed to disrupt the thallus and shear the cells. Loosely packed mycelia can be homogenized with 1.7 mm zirconium beads, while solid fruiting bodies can be disrupted with 2.8 mm stainless steel balls. It is possible that fruiting bodies and other complex structures like lichens might require a mixture of beads and balls, much like environmental samples. Low binding beads can be used with dilute cultures to minimize loss of analytes. Depending upon the beat beater, samples can be processed in 2 ml disruption tubes, deep well plates, 4 ml polycarbonate or polyethylene vials, and 15 ml polycarbonate vials.

Procedures

1. Filamentous fungi can be difficult to sample as they tend to grow best as pellicles and on solid media. Some fungi will grow in liquid culture but, generally, not as dispersed cells. Consequently, mycelium must be collected as a mass of cells for homogenization. Tightly packed mycelia can be cut with scissors or a scalpel, while fungi cultured in broth

can be collected by centrifugation or filtration. Pellicles can often be collected by plucking these from liquid cultures or scraping off membrane filters.

2. Prepare samples according to the following table:

Sample Type		Disruption Tubes (2 ml)	Deep Well Plates (square wells)	4 ml Vials	15 ml Vials
Mycelium	Pellet Size Bead Size Bead Mass	50 µl 800 µm Zr 600 mg	50 µl 800 µm Zr 600 mg	Up to 10 ml 800 µm Zr 1200 mg	Up to 50 ml 800 µm Zr 3 gm
Pellicle	Wet Weight Bead Size Bead Mass	200 mg 1.7 mm Zr 570 mg	200 mg 1.7 mm Zr 570 mg	500 mg 1.7 mm Zr 1200 mg	Up to 2 gm 1.7 mm Zr 3 gm
Thallus	Wet Weight Ball Size Number of Balls	200 mg 2.8 mm SS 8	200 mg 2.8 mm SS 8	500 mg 2.8 mm SS 15	Up to 2 gm 2.8 mm SS 30
Processing Time		2-5 minutes	2-5 minutes	5 minutes	5-10 minutes
Buffer Volume		600 µl	600 µl	1.5 ml	6 ml
Bead Beater Speed		High	High	High	High

3. Process the samples as stated in the table above. If pellicles are very dense, process as if they were a thallus, using 2.8 mm stainless steel balls. For heat sensitive samples, the processing can be divided into short bursts followed by a cooling period.
4. Sample homogenization can be monitored visually using a microscope. Hyphae should appear highly fragmented. Thallus should appear as a homogeneous slurry. If the sample is inadequately homogenized, repeat the processing and reassess the effectiveness.
5. Lysate can then be processed as needed.

Suggested Products

Beads: 800 µm Zirconium (BAWZ 800-250-30) for mycelia; 1.7 mm Zirconium (BAWZ 1700-300-22) for fungal balls, and 2.8 mm stainless steel balls (GBSS 089-1000-07) for thallus (e.g., mushroom)

Pre-filled Tubes: 800 µm Zirconium (PFAW 400-100-30) for mycelia; 1.7 mm Zirconium (PFAW 200-100-03) for pellicle, and 2.8 mm stainless steel (PFSS 2800-50-20) for thallus

Homogenization Vessel: See Appendix A

Homogenizers: See Appendix A

PLANTS

Pollen Homogenization

Pollen has a rigid cell wall that is most efficiently disrupted with zirconium beads. As the size range of pollen is very broad, bead size must be matched to the type of pollen. Undersized beads will have insufficient mass to crack open pollen, while using large beads will result in too few collisions. Pollen less than 10 µm in size should be disrupted with 200 µm zirconium beads, while pollen 10-50 µm in size should be processed with 400 µm zirconium beads. Large pollen (≥50 µm) is most effectively homogenized with 800 µm zirconium beads. Depending upon the bead beater, samples can be processed in disruption tubes, deep well plates, 4 ml polycarbonate or polyethylene vials, and 15 ml polycarbonate vials.

Procedures

1. Pollen can be effectively disrupted by bead beating, though the choice of beads is dependent on the size of the pollen granules. Pollen can be collected as a dry powder and weighed for processing. The beads used to bead beat pollen are 100 µm zirconium (for pollen <10 µm), 400 µm zirconium (for pollen 10-50 µm), and 800 µm zirconium (for pollen larger than 50 µm).

2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	Deep Well Plates (square wells)	4 ml Vials	15 ml Vials
Sample Mass	10 mg	10 mg	Up to 25 mg	Up to 100 mg
Buffer Volume	500 µl	500 µl	1 ml	5 ml
Bead Volume	400 µl	300 µl	1 ml	3 ml
Processing Time	2-5 minutes	2-5 minutes	5 minutes	5-10 minutes
Bead Beater Speed	High	High	High	High

3. Process the samples as stated in the table. For heat sensitive samples, the processing can be divided into short bursts followed by a cooling period.
4. Ruptured pollen grains appear with a crack or split. Visual inspection can be used to estimate homogenization efficiency using a microscope (number of cracked grains/total grains in the field of view). If the efficiency is less than 60%, continue to homogenize the sample.
5. Lysate can then be processed as needed.

Suggested Products

Beads: 100 µm Zirconium (BAWZ 100-250-15), 400 µm Zirconium (BAWZ 400-250-35), 800 micron Zirconium (BAWZ 800-250-30)
Pre-filled Tubes: 100 µm Zirconium (PFAW 100-100-02), 400 micron Zirconium (PFAW 400-100-30), 800 µm Zirconium (PFAW 800-100-29)
Homogenization Vessel: See Appendix A
Homogenizers: See Appendix A

Plant Leaf Tissue Homogenization

Leaf tissue can easily be homogenized by bead beating. Leaf punches, made with a common paper or Harris punch, are commonly used for genetic analysis. Small samples up to 50 mg (about five 6 mm leaf punches) can be processed in polypropylene deep well plates with one 5/32" stainless steel or zirconium oxide grinding ball per well; these samples can also be processed in disruption tubes with 1.0- 3.0 mm zirconium beads or 2.8 mm stainless steel balls. Denser leaf tissue with heavy cuticle will require larger beads and balls.

Samples with masses above 100 mg are best homogenized in grinding vials. Processing of up to 200 mg of leaf tissue is best done in 4 ml polycarbonate vials with one 5/16" or 3/8" grinding ball. Up to a gram of leaf tissue can be homogenized in 15 ml polycarbonate vials with two 7/16" stainless steel grinding balls. Both 4 ml and 15 ml vials can be purchased as Vial Sets, meaning that they are packaged in a rack with stainless steel grinding balls and caps. Zirconium oxide is an alternative to stainless steel grinding balls, which may react when used with corrosive buffers.

Leaf tissue is often a source of genomic DNA, and the method chosen to grind it will significantly affect the size of the isolated fragments. Bead beating in solution will generate smaller DNA fragments than dry or cryogenic grinding. Smaller fragments are adequate for use in many applications, such as PCR, but if high molecular weight DNA is required, then leaf tissue should be either lyophilized prior to grinding or homogenized cryogenically. Cryoblocks, which keep samples frozen and dissipate heat during processing (see Cryogenic Homogenization above), are typically used for cryogenic grinding.

Procedures

1. One leaf punch is approximately 10 mg (depending upon the plant). Multiple punches can be placed in one tube or larger pieces of leaf can be weighed and added to vessels. Grinding balls are most effective, though larger size beads can be used if available.

2. If dry grinding lyophilized leaf, omit the buffer. For cryogenic grinding, use either 4 ml or 15 ml polycarbonate vials and omit the buffer. Deep well plates and disruption tubes will often crack if used for cryogenic grinding. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	Deep Well Plates (square wells)	4 ml Vials	15 ml Vials
Sample Size	50 mg	50 mg	200 mg	Up to 1 gm
Buffer Volume	600 µl	600 µl	2 ml	6 ml
Grinding Balls	8 x 2.8 mm	1 x 5/32”	1 x 3/8”	2 x 7/16”
Processing Time	1 minute	1 minute	2 minutes	2-5 minutes
Bead Beater Speed	2/3 speed	2/3 speed	2/3 speed	2/3 speed

3. Process the samples as stated in the table. Processing can be divided into short bursts followed by a cooling period for heat sensitive samples not processed cryogenically.
4. Check the homogenate following processing. Typically a well processed sample will appear as a smooth green liquid. If large pieces of leaf tissue are present, repeat the processing.
5. Sample can be stored or processed as needed.

Suggested Products

Balls: 2.8 mm Stainless Steel Grinding Balls (GBSS 089-1000-07), 5/32” Stainless Steel Grinding Balls (GBSS 156-5000-01), 3/8” Stainless Steel Grinding Balls (GBSS 375-1000-02), 7/16” Stainless Steel Grinding Balls (GBSS 412-1000-03)
Pre-filled Tubes: 3.0 mm Zirconium (PFAW 3000-50-17), 2.8 mm Stainless Steel (PFSS 2800-50-20)
Disruption Tube: 2 ml Skirted Disruption Tubes (PPDT 02-500-13)
Vial Sets: 24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vial Sets (PCVS 15-050-22)
Homogenization Vessel: See Appendix A
Homogenizers: See Appendix A

Plant Stem Homogenization

Stems are characterized by a high concentration of vascular tissues that can be resistant to shearing and grinding. Small young stems are soft and can be processed much like leaves. Plant stem masses less than 50 mg can be homogenized in polypropylene deep well plates with one 5/32” stainless steel grinding ball per well; they can also be processed in disruption tubes with 1.7- 3.0 mm zirconium beads or 2.8 mm stainless steel balls.

Unlike the softer stems of young plants, tougher, larger stem samples are best homogenized in 4 ml or 15 ml grinding vials. Processing up to 200 mg of stem tissue is best done in 4 ml polycarbonate or polypropylene vials with one 5/16” or 3/8” stainless steel grinding ball. Up to one gram of stem tissue can be homogenized in a 15 ml polycarbonate vial with two 7/16” stainless steel grinding balls. Zirconium oxide grinding satellites are resistant to corrosive chemicals such, as phenol, and can be used as a substitute for stainless steel in order to prevent reactions that may interfere with sample processes.

Procedures

1. Stem can be homogenized with buffer, dry ground following lyophilization or bead beaten cryogenically. Dry grinding and cryogenic grinding are done without buffer, which can be added after bead beating. Cryogenic grinding should be done in 4 or 15 ml polycarbonate vials, as polypropylene tubes and plates may crack during cryogenic grinding.

2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	Deep Well Plates (square wells)	4 ml Vials	15 ml Vials
Sample Size	50 mg	50 mg	200 mg	Up to 1 gm
Homogenization Buffer Volume	600 µl	600 µl	2 ml	6 ml
Grinding Balls	8 x 2.8 mm	1 x 5/32"	1 x 3/8"	2 x 7/16"
Processing Time	2 minutes	2 minutes	2 minutes	2-5 minutes
Bead Beater Speed	high speed	high speed	high speed	high speed

3. Process the samples as stated in the table. Processing can be divided into short bursts followed by a cooling period for heat sensitive samples.
4. Samples should be processed until a homogenate is generated, i.e., fine powder for dry and cryogenic grinding, or a fine puree for wet grinding.
5. Sample can be stored or processed as needed.

Suggested Products

Balls: 2.8 mm Stainless Steel Grinding Balls (GBSS 089-1000-07), 5/32" Stainless Steel Grinding Balls (GBSS 156-5000-01), 3/8" Stainless Steel Grinding Balls (GBSS 375-1000-02), 7/16" Stainless Steel Grinding Balls (GBSS 412-1000-03)
Pre-filled Tubes: 2.8 mm Stainless Steel (PFSS 2800-50-20)
Disruption Tube: 2 ml Skirted Disruption Tubes (PPDT 02-500-13)
Vial Sets: 24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vials (PCVS 15-050-22)
Homogenization Vessel: See Appendix A
Homogenizers: See Appendix A

Seed Homogenization

Seeds are best homogenized via dry grinding by using a disproportionally large grinding ball to crack these hard samples. Some protocols call for freeze drying the seeds prior to bead beating, but this is not always necessary. If seeds are air dried before homogenization, then water content may be sufficiently low to allow for the generation of a fine meal.

The choice of grinding vessel is very important when bead beating seeds. Most plastics tend to soften as the tube heats during processing; therefore polycarbonate should be used to ensure a hard grinding surface during homogenization. In almost all instances, 5/32" grinding balls are too small to effectively process seeds. For instance, a single grain of rice requires no less than a 3/8'' stainless steel grinding ball and polycarbonate vial. Single seeds are commonly processed in 4 ml grinding vials with one 3/8" grinding ball to ensure that the seed is pulverized. Multiple seeds should be processed in 15 ml polycarbonate vials with two 7/16" stainless steel grinding balls. Up to fifteen corn kernels (about 5 gm) can be ground in a 15 ml vial. In the event that stainless steel balls do not have enough energy to pulverize a seed, tungsten carbide grinding balls can be substituted. Some seeds, such as palm nuts, are virtually impossible to process by bead beating.

Procedures

1. Seeds should be dry ground. Extraction buffers can be added after grinding. Grinding should be done in 4 or 15 ml polycarbonate vials with larger grinding balls.
2. Prepare samples according to the following table:

	4 ml Vials	15 ml Vials
Sample Size	One seed	Up to 5 gm
Grinding Balls	1 x 3/8"	2 x 7/16"
Processing Time	2-3 minutes	3-5 minutes
Bead Beater Speed	high speed	high speed

3. Process the samples as stated in the table. Usually heat is not a big issue in dry grinding. However, should it become a problem, sample processing can be divided into short bursts followed by a cooling period.
4. Samples should be processed until a powder is generated. Ideally the powder will be fine in texture, but moisture present in the seed may make the powder mealy.
5. Sample can be processed as needed.

Suggested Products

Balls:	3/8" Stainless Steel Grinding Balls (GBSS 375-1000-02), 7/16" Stainless Steel Grinding Balls (GBSS 412-1000-03)
Vial Sets:	24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vials (PCVS 15-050-22)
Homogenization Vessel:	See Appendix A
Homogenizers:	See Appendix A

ANIMAL

Soft Animal Tissue Homogenization: Liver/Brain

Animal tissues, such as blood, liver, brain and thymus, are easily homogenized by bead beating. The small amount of connective tissue allows for these soft tissues to be processed very efficiently. Soft animal tissues can be homogenized with a buffer or cryogenically, the latter method being more useful for isolating high molecular weight DNA. Samples less than 100 mg can be processed in disruption tubes with 1.4-3.0 mm zirconium beads. Smaller samples can also be homogenized in polypropylene deep well plates with one 5/32" grinding ball or 6 mm satellite per well.

Larger samples are best homogenized in grinding vials. Processing up to 200 mg of animal tissue is best done in a 4 ml polycarbonate vial with one 5/16" or 3/8" stainless steel grinding ball. Up to two grams of animal tissue can be homogenized in a 15 ml polycarbonate vial and two 7/16" stainless steel grinding balls. In both instances, grinding can be done with buffer, or cryogenically without buffer. Cryogenic grinding is best done using Cryoblocks to prevent excessive melt back of the sample. Polyethylene vials should be used instead of polycarbonate when using extraction buffers containing phenol and/or chloroform. Zirconium oxide grinding satellites are chemically resistant and can be used with corrosive extraction buffers.

Procedures

1. Soft animal tissue can be processed in many different formats, though square deep well plates should be avoided. When bead beating with homogenization buffer, do not to overfill tubes with sample and buffer as homogenate can be very thick. For cryogenic grinding, omit buffer during the processing. Buffers can be added after grinding, or the sample can be left frozen (and archived) until needed.
2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	Deep Well Plates (Round wells)	4 ml Vials	15 ml Vials
Sample Mass	20 mg	20 mg	100-200 mg	Up to 2 gm
Buffer Volume	600 µl	200 µl	1 ml	6 ml
Grinding Balls	8 x 2.8 mm	1 x 5/32"	1 x 3/8"	2 x 7/16"
Processing Time	2 minutes	2 minutes	2-3 minutes	3-5 minutes
Bead Beater Speed	High	High	High	High

3. Process the samples as stated in the above table. Processing can be divided into short bursts followed by a cooling period for heat sensitive samples.
4. Sample homogenization can be monitored visually. A homogenate with a smooth appearance and lack of solids is a desirable end product. Disruption efficiency can be measured using a marker enzyme, such as lactate dehydrogenase, which is liberated from cells during processing.
5. Lysate can be stored or processed as needed.

Suggested Products

Balls:	2.8 mm Stainless Steel (GBSS 089-100-07), 5/32'' Stainless Steel (GBSS 156-5000-01), 3/8'' Stainless Steel (GBSS 375-1000-02), 7/16'' Stainless Steel (GBSS 437-1000-03)
Pre-filled Tubes:	2.8 mm Stainless Steel (PFSS 2800-50-20)
Disruption Tube:	2 ml Skirted Disruption Tubes (PPDT 02-500-13)
Vial Sets:	24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vial Sets (PCVS 15-050-22)
Homogenization Vessel:	See Appendix A
Homogenizers:	Cryogenic grinding requires a GenoGrinder® or Mini G™ (Appendix A)
Cryogenic Option:	Cryoblocks (4 ml and 15 ml)

Fibrous Animal Tissues: Muscle/Heart/Lung

Animal tissues, such as muscle, heart and lung, require substantially greater force to homogenize than softer tissues because of significant amounts of connective tissue and microfilaments. Small whole animal samples, such as *Drosophila* and *C. elegans*, can be treated as small fibrous samples. Fibrous tissue can be homogenized with buffer or cryogenically, the latter being useful for high molecular weight DNA isolation.

Samples less than 50 mg can be processed in disruption tubes with 1.7-3.0 mm zirconium beads or small stainless steel balls. Small samples (10-50 mg) can also be homogenized in polypropylene deep well plates with one 5/32" grinding ball or 6 mm satellite per well.

Larger samples should be homogenized in grinding vials. Processing up to 200 mg of animal tissue is best done in a 4 ml polycarbonate vial with one 3/8" stainless steel grinding ball. Up to two grams of animal tissue can be homogenized in a 15 ml polycarbonate vial with two 7/16" stainless steel grinding balls. Polyethylene should be used over polycarbonate for samples that require organic solvents (phenol/chloroform buffers) that melt polycarbonate. Zirconium oxide grinding satellites are resistant to corrosive chemicals such as phenol and can be used with garnet shards to rip and cut the tissue.

Procedures

1. Fibrous animal tissue can be processed in many different formats, though square deep well plates should be avoided. When bead beating with homogenization buffer, do not to overfill tubes with sample and buffer; it is important that the ball move freely during processing. For cryogenic grinding, omit buffer during the processing. Buffers can be added after grinding, or the sample can be left frozen until needed.
2. Prepare samples according to the following table:

	Disruption Tubes (2 ml)	**Deep Well Plates (Round wells)**	**4 ml Vials**	**15 ml Vials**
Sample Mass	20 mg	20 mg	100-200 mg	Up to 2 gm
Buffer Volume	600 µl	200 µl	1 ml	6 ml
Grinding Balls	8 x 2.8 mm	1 x 5/32"	1 x 3/8"	2 x 7/16"
Processing Time	2 minutes	2 minutes	3-5 minutes	5-10 minutes
Bead Beater Speed	High	High	High	High

3. Process the samples as stated in the table. For heat sensitive samples, the processing can be divided into short bursts followed by a cooling period.
4. Sample homogenization can be monitored visually. A homogenate with a smooth appearance and lack of solids is a desirable end product. Using a microscope, muscle fibers should be highly fragmented, although complete disruption does not usually occur. Disruption efficiency can be measured using a marker enzyme, such as lactate dehydrogenase, which is liberated from cells during processing.
5. Lysate can be processed as needed.

Suggested Products

Balls:	2.8 mm Stainless Steel (GBSS 089-100-07), 5/32'' Stainless Steel (GBSS 156-5000-01), 3/8'' Stainless Steel (GBSS 375-1000-02), 7/16'' Stainless Steel (GBSS 437-1000-03)
Pre-filled Tubes:	2.8 mm Stainless Steel (PFSS 2800-50-20)
Disruption Tube:	2 ml Skirted Disruption Tubes (PPDT 02-500-13)
Vial Sets:	24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vials (PCVS 15-050-22)
Homogenization Vessel:	See Appendix A
Homogenizers:	Cryogenic grinding requires a GenoGrinder® or Mini G™. See Appendix A
Cryogenic Option:	Cryoblocks (4 ml and 15 ml)

Elastic Animal Tissue Homogenization: Skin/Sclera/Cartilage

Animal tissues that contain a significant amount of collagen such as skin, sclera, and cartilage are very difficult to homogenize by any method. The pliable and resilient nature of these tissues is by design. In order to effectively homogenize these elastic tissues, the mass of the sample needs to be very small relative to the grinding media and vessel. Samples less than 20 mg are best processed in a 4 ml polycarbonate vial with one 3/8" stainless steel ball. Larger samples are best processed in a 15 ml polycarbonate vial with two 7/16" stainless steel grinding balls. It is often necessary to homogenize elastic tissues cryogenically. In such cases, a GenoGrinder® or Mini G™ models equipped with Cryoblocks are needed.

Procedures

1. The grinding formats available for elastic tissues are limited. Sample size is also limited. Samples need to be dissected into small pieces (3 x 3 mm) prior to homogenization. Avoid thick, large pieces of tissue since these will not effectively homogenize. Samples are best processed cryogenically using liquid nitrogen to chill the sample, tube, ball and Cryoblock. Caps are usually made of polypropylene and, if too cold, will crack.
2. Prepare samples according to the following table:

	4 ml Vials	15 ml Vials
Sample Mass	20 mg	Up to 250 mg
Grinding Balls	1 x 3/8"	2 x 7/16"
Processing Time	5 minutes	5-10 minutes
Bead Beater Speed	High	High

3. Process the samples as stated in the table. Heat is generated during processing, so it is possible that samples may warm and thaw by the end of processing.
4. Sample homogenization can be monitored visually. Ideally the tissue will be converted into a fine powder. Samples with large amounts of collagen may not homogenize well.
5. Homogenized tissue can be stored frozen or thawed and used.

Suggested Products

Balls:	3/8'' Stainless Steel (GBSS 375-1000-02), 7/16'' Stainless Steel (GBSS 437-1000-03)
Vial Sets:	24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vial Sets (PCVS 15-050-22)
Homogenization Vessel:	See Appendix A
Homogenizers:	Cryogenic grinding requires a GenoGrinder® or Mini G™. See Appendix A
Cryogenic Option:	Cryoblocks (4 ml and 15 ml)

Hard Animal Tissues: Bone/Hair/Nail

Hard animal tissues such as bone, hair, fish scale, and nail are best homogenized using a disproportionally large grinding ball to crack the sample. Most hard tissues are ground cryogenically, using liquid nitrogen to make substances extra brittle. Hair is very fine, making it difficult to grind into a fine powder, while nail is more resilient and requires cryogenic temperatures for homogenization. Bone, however, can be homogenized with buffer because it is naturally brittle when compared to hair and nail.

The size and mass of samples will influence which ball size, bead beater and configuration one should use. As homogenization is the result of crushing the sample between a grinding balls and vessel wall, a hard plastic (such as polycarbonate) is needed. Therefore, samples less than 100 mg are best homogenized in a 4 ml polycarbonate vial with one 3/8" stainless steel or tungsten carbide ball. Larger samples should be processed in a 15 ml polycarbonate vial with two 7/16" stainless steel grinding balls. In

the event that stainless steel balls do not have enough energy to pulverize the sample, tungsten carbide grinding balls can be substituted.

Procedures

1. Hard animal tissue requires grinding vials made of polycarbonate. If bead beating at ambient temperatures, do not overfill tubes with buffer. For cryogenic grinding, omit buffer during the processing. Buffers can be added after grinding, or the sample can be left frozen (and archived) until needed.
2. Prepare samples according to the following table:

	4 ml Vials	**15 ml Vials**
Sample Mass	100 mg	Up to 1 gm
Buffer Volume	1 ml	6 ml
Grinding Balls	1 x 3/8" SS	2 x 7/16" SS
Processing Time	3-5 minutes	5-10 minutes
Bead Beater Speed	High	High

3. Process the samples as stated in the table. During longer processing runs, sample may warm and melt.
4. Sample homogenization can be monitored visually. For cryogenic grinding, homogenized sample should appear as a fine powder. With bone, a homogeneous solution is an indication of an effective process. Small flakes of bone may often be present in the bottom of the tube.
5. Frozen samples can be stored. Alternatively, samples can be processed immediately after grinding.

Suggested Products

Balls:	3/8'' Stainless Steel (GBSS 375-1000-02), 7/16'' Stainless Steel (GBSS 437-1000-03)
Vial Sets:	24 Well PC Vial Set (PCVS 04-240-03), 15 ml Polycarbonate Vial Sets (PCVS 15-050-22)
Homogenization Vessel:	See Appendix A
Homogenizers:	Cryogenic grinding requires a GenoGrinder® or Mini G™. See Appendix A
Cryogenic Option:	Cryoblocks (4 ml and 15 ml)

Appendix A – Homogenizer Capacity and Configuration

	Vortexer	HT Mini™	HT 24™	HT Homogenizer™	1600 Mini G™	2010 GenoGrinder®
Characteristics						
Motion	Vortex	Arch	Figure 8	Linear	Linear	Linear
Speed - High	3000 rpm	4000 rpm	4200 rpm	1600 rpm	1500 rpm	1750 rpm
Speed - Low	Varies	2800 rpm	2400 rpm	50 rpm	500 rpm	500 rpm
Timer	Available	0-60 sec.	0-90 sec[4]	0-10 min.	0-10 min.	0-20 min.
Pulsing Feature	Available	No	Yes	No	No	No
Cryogenic Grinding	No	No	No	No	Yes	Yes
Grinding Formats						
Microcentrifuge Tubes	12	-	-	24	50	100
Disruption Tube (nonskirted)	-	3	24	24	50	100
Disruption Tube (skirted)	-	-	24	24	50	100
Deep Well Plate	-	-	-	1	2	4-6[5]
4 ml Grinding Vials	-	-	-	24	48	144
15 ml Grinding Vials	-	-	-	5	10	30
15 ml Conical Tubes	-	-	-	-	12	24
50 ml Conical Tubes	-	-	-	-	6	12

Appendix B – Cryogenic Homogenization

Cryogenic homogenization can be very useful for disrupting highly resilient samples, or when isolating biochemicals that are extremely sensitive to thermal or enzymatic degradation. The traditional method of cryogenically grinding materials involves the use of a mortar and pestle placed in a liquid nitrogen bath. With bead beating, materials can be placed in a bath to chill, but homogenization vessels, grinding media, and sample must be removed from the bath for processing. Depending upon the processing conditions, it is very common for samples to warm and melt. Consequently, Cryoblocks were designed to act as a cold reservoir during processing in order to keep samples frozen.

Though Cryoblocks are not mandatory for cryogenic homogenization, they are very useful. Cryoblocks are solid rectangular bars of aluminum that have been machined to hold 4 ml and 50 ml polycarbonate vials. The blocks have essentially the same footprint as a 96 well plate. Prior to sample processing, Cryoblocks are placed in a CryoCooler™ or similar device and chilled with liquid nitrogen. Vials are loaded into the blocks (vials with sample can be pre-frozen) along with sample and allowed to chill. Balls are then dropped on top of the samples and also allowed to chill. Caps, however, are not pre-chilled because they are made of polypropylene and will become very brittle if chilled to cryogenic temperatures.

The vials are capped just prior to bead beating. The Cryoblock is removed from the CryoCooler and placed in the homogenizer. Only the GenoGrinder® and 1600 Mini G™ are capable of holding Cryoblocks. The GenoGrinder® can hold two while the 1600 Mini G™ can hold one. Processing the samples should yield a powder. Return the Cryoblock and samples back to the CryoCooler so that the samples do not melt.

[4] The HT 24 can run for 90 seconds continuously, but can cycle 10 times using the pulsing feature. When pulsing, a pause is inserted between the run times, and the pause is between 1and 120 seconds. Thus, total processing time can be up to 15 minutes with pauses of as little as 1 second.

[5] Initially the GenoGrinder 2010 could be adapted to hold four deep well plates. The clamp assembly was later modified to hold up to six plates.

Suggested Products

Homogenizers:	1600 Mini G™ (SP 1600)
	2010 GenoGrinder® (SP 2010-115)
Cryoblocks:	24 Well Cryoblock (SP-2662)
	15 ml Cryoblock (SP-2660)
Polycarbonate Vials:	4 ml Polycarbonate Replacement Vials (PCRV 04-240-10)
	15 ml Polycarbonate Replacement Vials (PCRV 15-100-15)
Grinding Balls:	3/8" Stainless Steel Grinding Balls (GBSS 375-1000-02)
	7/16" Stainless Steel Grinding Balls (GBSS 437-1000-03)
Cryogenic Reservoir:	CryoCooler™ (CG 08-07)

Appendix C – Generic Buffers and Downstream Protocols

Since many researchers use commercial kits for the harvesting of proteins and nucleic acids from samples, the protocols listed above provide no specific details about homogenization buffers. In most cases, the first buffer used for isolating DNA or RNA can be used as the homogenization buffer. Though fewer commercial kits are available for protein isolation, the same is essentially true. For those researchers on a tight budget or in a spot (e.g., it is a weekend and you need that molecule), here are a couple of basic homogenization buffer recipes and related protocols. Some reagents used in these protocols are dangerous, so exercise caution when using them.

Protein Isolation

Proteins are typically isolated as active molecules or in a denatured state. For best yields of native proteins, homogenization buffers should contain protease inhibitors, and the process should be done at 4˚C. The final homogenate should also be refrigerated, as freezing of protein can lead to its denaturation. Alternatively, proteins can be isolated using buffers containing detergents such as SDS. The detergent denatures the proteins, including proteases, and prevents enzymatic breakdown. It must be noted that homogenization buffers with detergent will foam, which can hinder the movement and effectiveness of grinding beads and balls during beating.

Native Homogenization Buffer: 100 mM Tris, pH 7.6, 20 mM glutathione (DTT or 2-mercaptoethanol can be used), 5% sucrose, 2 mM EDTA (ethylenediaminetetraacetic acid), 1 mM PMSF (phenylmethanesulfonyl fluoride [this is toxic]), 5 µM Pepstatin A. Many different protease inhibitors can be added to homogenization buffers, many of which are not readily available. EDTA inhibits many metallo-proteases while PMSF acts against many serine and cysteine proteases. At a minimum, EDTA and PMSF should be included in the buffer. If the protein solution is to be used for assays involving formazan dyes, leave out the glutathione.

Protocol

1. Add sample, buffer, and beads/balls to the disruption vessel. Keep cold until processing
2. Fix the homogenization vessel into the homogenizer and bead beat. Multiple short processing times can be used if excessive heat is a concern.
3. Remove the vessels from the homogenizer and centrifuge the contents at high speed. This will remove insoluble cellular debris from the homogenate.
4. Transfer the supernatant to a clean tube. Refrigerate the sample lysate until needed. Do not freeze the solution if there are concerns about activity loss due to denaturation.

Denaturation Buffer: 50 mM Tris, pH 7.5, 150 mM NaCl, 1.0% (v/v) NP-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecylsulfate.

Protocol

1. Place sample, buffer, and grinding ball in vessel. Small beads may not work well with this buffer due to foaming.
2. Process samples in a homogenizer. Heat buildup is not an issue as the buffer is denaturing.
3. Centrifuge the homogenate to pellet insoluble debris. Transfer cleared supernatant to a clean tube.
4. Sample can be refrigerated or stored frozen.

DNA Isolation from Animal Tissues

The separation of DNA from other biomolecules in lysates is traditionally accomplished by exploiting DNA solubility characteristics. In animal tissues, DNA can be released from the cell by bead beating either cryogenically or in solution. Homogenization buffers usually contain Tris buffer, EDTA, SDS, and Proteinase K. SDS can foam extensively during bead beating and can be added after sample processing. Though most methods used to isolate DNA employ silica spin columns (usually part of a kit), the protocol below uses a traditional method that relies upon spooling the DNA onto glass rods or Pasteur pipettes.

Homogenization Buffer: 50 mM Tris, pH 9, 100 mM EDTA, 200 mM NaCl.

Other Solutions: 10% SDS, 10 mg/ml Proteinase K in water, TE buffer (10 mM Tris, pH 8, 1 mM EDTA), phenol/chloroform/isoamyl alcohol (in a ratio of 25:24:1) stored under TE buffer (DO NOT GET THIS SOLUTION ON YOUR SKIN AS IT BURNS!), 3M sodium acetate, 95% ethanol (cold)

Protocol

1. Depending upon the use of the DNA, samples may best be homogenized cryogenically and then suspended in homogenization buffer. If high molecular weight DNA is needed, homogenize the samples cryogenically. Alternatively, samples can be homogenized in the buffer and then supplemented with SDS and Proteinase K.
    a. Cryogenic Grinding – Following cryogenic bead beating, add 8 volumes of homogenization buffer as compared to the mass of the sample (i.e., if the sample is 100 mg, add 0.8 ml). Add 1 volume of each 10% SDS and 10 mg/ml Proteinase K. Mix and incubate at 55˚C for 1 hr.
    b. Solution Grinding – Add sample and buffer to a vessel along with beads/balls. Process the sample as described. Open the vessel and add 1/8 volume (original buffer volume) of each 10% SDS and 10 mg/ml Proteinase K. Mix and place at 55˚C for 1 hr.
2. Add an equal volume of phenol/chloroform/isoamyl alcohol to the homogenate. Cap the vessels and mix by gently inverting. Centrifuge to separate the phases. The aqueous phase containing the DNA is on the top. Carefully remove the aqueous phase with a polypropylene pipette tip and transfer to a clean tube.
3. Add 1/10 volume of 3 M sodium acetate and mix. Using ice-cold ethanol, carefully layer two (2) volumes of the ethanol over the DNA solution. A cloudy phase will form on the interface of the ethanol and aqueous phase. This is the where the DNA is spooled onto glass.
4. Using a clean Pasteur pipette, insert the tip into the cloudy interface and slowly twirl. DNA will stick to the glass. Remove the spooled DNA from the tube and wash off the Pasteur pipette with a small volume of TE buffer (minimize this volume). If the DNA did not spool onto the glass, then continue on to step 5.
5. If the DNA was excessively sheared during homogenizing, it may not spool. Thus continuing, the tube with ethanol can be mixed and placed in a -20˚C freezer for 1 hr to allow DNA to precipitate. Centrifuge the DNA solution at 10,000 x g for 20 minutes. Decant and wash the pellet with 70% ethanol to remove residual salt. Air dry the pellet, but do not allow the pellet to become dry (the goal is to remove extraneous alcohol).
6. Resuspend the pellet in a small volume of TE buffer (e.g., 20 µl). Store at -20˚C until needed.

Isolation of Total RNA from Animal Tissues

RNA can be relatively stable within cells, especially tissues, but once released into a homogenate, it can be enzymatically degraded by RNases quickly. RNases are ubiquitous and must be rendered inactive to protect RNA during sample processing. This is accomplished by disrupting samples under conditions that inactivate the RNases (either cryogenically or with chemical denaturation) and then removing the RNases using organic extractions. Phenol is used to remove protein, including RNases, while the chloroform/isoamyl alcohol removes residual phenol from the RNA solution. As this process requires the use of phenol (which can cause serious chemical burns) and chloroform (carcinogenic), use extreme caution when performing this protocol. It is best to do the extractions in a chemical hood.

Homogenization Buffer: 4 M Guanidine thiocyanate, 42 mM citrate pH 4.3, 0.1% N-lauroylsarcosine, 0.1 M 2-mercaptoethanol

Isolation Reagents: Phenol saturated (stored under) 42 mM citrate buffer, pH 4.3, chloroform/isoamyl alcohol (ratio of 24:1), 3 M sodium acetate, pH 5.2, 95% ethanol (store at -20˚C), 70% ethanol (store at -20˚C), water (molecular biology grade).

Protocol

1. Place the sample, homogenization buffer and grinding media in a grinding vessel and homogenize by bead beating. Alternatively, tissue can first be pulverized cryogenically, with homogenization buffer added to the powdered tissue subsequently. In either case, centrifuge the homogenate to pellet insoluble cellular debris and transfer the supernatant to a clean polypropylene tube.
2. Add an equal volume of buffer saturated phenol, vortex for 30 sec., than add an equal volume of chloroform/isoamyl alcohol. Vortex for 30 seconds, and centrifuge for 5 min. to separate the phases.
3. Transfer the upper aqueous phase containing the RNA to a clean tube.
4. Repeat the phenol/chloroform extraction again and transfer the supernatant to a clean tube.
5. Add 1/10 volume of 3 M sodium acetate, pH 5.2. Mix and add 2.5 volumes of 95% ethanol and mix. Incubate at -20˚C for 30 minutes to overnight.
6. Centrifuge for 15 minutes (12,000 g or on high in microfuge) to pellet the RNA.
7. Decant and wash the pellet with 70% ethanol. Centrifuge for 1 minute, decant, and air dry pellet.
8. Resuspend the RNA in molecular biology grade water and store at -80˚C.

DNA Isolation from Plants

Isolating DNA from plant homogenates is often complicated by the presence of polysaccharides and polyphenolics. Homogenization buffers containing CTAB (cetyltrimethylammonium bromide) and PVP (polyvinylpyrrolidone) are commonly used to remove polysaccharides and polyphenolics, respectively. Polysaccharides will precipitate in solutions containing CTAB and sodium chloride, while PVP binds polyphenolics.

Homogenization Buffer: 2% cetyltrimethylammonium bromide (CTAB), 1.4 M NaCl, 0.1M Tris/HCl, 20 mM EDTA

Isolation Reagents: CTAB precipitation solution (0.5% CTAB, 0.04 M NaCl), chloroform/isoamyl alcohol (ratio of 24:1), 1.2 M NaCl, isopropanol, TE buffer (10 mM Tris, pH 8, 1 mM EDTA)

Protocol

1. Sample can be homogenized cryogenically, dry, or with buffer, depending upon the nature of the sample. Cryogenic grinding typically yields the largest DNA fragments. Whether added before or after grinding, use 1 ml homogenization buffer for every 50 mg of tissue. Incubate for 30 minutes at 65°C.
2. Centrifuge the mixture for 10 minutes to pellet plant debris and clarify the solution. Transfer the supernatant to a new tube.
3. Add an equal volume of chloroform/isoamyl alcohol and mix the contents gently. Centrifuge for 10 minutes to separate the phases.
4. Transfer the upper phase to a clean tube and add two volumes of CTAB precipitation solution. Mix and incubate at room temperature for 60 minutes.
5. Centrifuge for 10 minutes at 12,000 g (or high on microfuge). Decant and dissolve the pellet in 1.2 M NaCl.
6. Extract the solution with an equal volume of chloroform/isoamyl alcohol. Centrifuge to separate the phases.
7. Transfer the upper aqueous phase to a new tube and add 0.7 volumes of isopropanol. Incubate at -20˚C for 30 minutes. Centrifuge at 12,000 g for 20 minutes.
8. Decant and air-dry the pellet. Resuspend the pellet in TE buffer.

RNA Isolation from Plants

In addition to RNases, polyphenolics and polysaccharides compound the difficulty of isolating RNA from plants. Consequently a combination of methods is used to protect the RNA while eliminating contaminants. RNA isolation is best accomplished using cryogenic homogenization.

Homogenization Buffer: 0.25 M NaCl, 50 mM Tris (pH 7.5), 20 mM EDTA, 1% SDS, 4% PVP

Isolation Reagents: Phenol saturated (stored under) with 42 mM citrate buffer, pH 4.3, chloroform/isoamyl alcohol (ratio of 24:1), 3 M sodium acetate, pH 5.2, 95% ethanol (store at -20˚C), 70% ethanol (store at -20˚C), and water (molecular biology grade). NOTE: Phenol can cause chemical burns and chloroform is carcinogenic. Exercise caution when working with these chemicals.

Protocol

1. Plant tissue should be flash frozen when harvested and placed in a polycarbonate grinding vial for cryogenic processing. Homogenize the sample cryogenically. Do not allow the sample to thaw.
2. Using a nuclease-free tube, add 750 µl of Homogenization Buffer and 750 µl chloroform/isoamyl alcohol for each 100 mg of homogenized plant tissue. Add the powdered plant tissue and vortex for 30 seconds.
3. Centrifuge the tube at 12,000 g to separate the phases. Transfer the aqueous phase (upper) to a clean tube.
4. Add an equal volume of buffer saturated phenol. Vortex for 30 seconds and centrifuge to separate the phases. Transfer the aqueous phase (upper) to a clean tube.
5. Repeat the phenol extraction and transfer the supernatant to a clean tube.
6. Add an equal volume of chloroform/isoamyl alcohol. Vortex, centrifuge, and transfer supernatant to a clean tube. Repeat this step.
7. Add 1/10 volume of 3 M sodium acetate and mix. Add 2.5 volumes of cold 95% ethanol. Mix and incubate at -20˚C for 30 minutes.
8. Centrifuge the sample at 12,000 g for 20 minutes. Decant and wash the pellet with cold 70% ethanol. Decant and air-dry the pellet.
9. Dissolve the pellCet in molecular biology grade water. Store at -80˚C.