



# HBS MOLECULAR LAB USER MANUAL

Welcome to the “Tree House”, the HBS’s new molecular lab! This manual is designed to give users detailed information about our equipment and provide suggestions about what users need to bring with them to complete molecular work. The information in this document pertains to DNA/RNA extraction, quantification, PCR, and visualization.

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## DNA/RNA EXTRACTION

### Equipment at HBS:

- **Mini-Beadbeater-16-cell disrupter** that accepts 2ml screw cap tubes and disrupts cells by shaking them with beads and lysis buffer to break up tissue. See their website [www.biospec.com](http://www.biospec.com) as a resource. Beadbeater can process up to 16 samples at a time.
- **Water bath**
- **Eppendorf Centrifuge 5415 C**-holds 24 tubes and spins up to 14,000rpm and can be stored in a refrigerator to perform precipitation at cold temps. (We also have an **Eppendorf Centrifuge 5430** in the PCR lab that can spin both tubes and plates).
- **Barnstead EASYpure II uv/uf water filtration system** - provides type 1 water appropriate for all DNA work.
- **Refrigerator, -20 freezers** (2 ultralow -80 freezers are available in the Coker Lab)
- **0.2 and 1.5ml tube racks**
- **Ice buckets**
- **Flake ice maker**
- **Glassware**
- **Fume hood**

### Supplies that users must bring:

1. 2ml screw top tubes with premixed beads. Kits preferred (recommendations below).
2. Lysis buffer/extraction kit
3. Additional reagents required in extraction protocol (organics, alcohols, etc.)
4. 1.5ml microfuge tubes
5. Pipette tips (filter tips recommended)
6. Sterile purified water (if desired)
7. Gloves
8. Lab tape and sharpies

### Notes on equipment:

#### **Mini-BeadBeater-16-cell disruptor**

HBS does not provide liquid Nitrogen, so in order to extract DNA we provide a Mini-BeadBeater-16 that disrupts cells and tissue by pulverizing the sample with beads. The machine has a vigorous shaking mechanism with room for up to 16 samples. Tissue must be loaded into 2 ml screw-cap microvials that contain the tissue, beads, and a disruption buffer. We strongly recommend using one of many Gu-SCN commercial kits that are compatible with the Mini-BeadBeater. The final product of these kits is PCR-ready DNA. These kits are listed on BioSpec's website (<http://biospec.com/content.php?id=1056>). We have successfully tested Mo-Bio's PowerPlant® DNA Isolation Kit using the Mini-BeadBeater-16 and found it to be easy and effective. Mo-Bio offers free sample kits of two preparations each (you can order up to three sample kits for a total of six preparations). We prefer it if users can select a non-toxic extraction protocol that does not require phenol or chloroform, although we do provide a fume hood and can help users dispose of liquid and solid organic wastes.

If you choose not to use a commercial kit, you must buy your own beads and tubes. Depending on the type of tissue you are working with, the hardness of the beads (and as a result, the durability of the tubes) will differ. Mo-Bio has a useful chart on its website for selecting the proper beads for your type of sample. This chart is posted below, as are BioSpec's guidelines for selecting beads. Bead types vary slightly between BioSpec and Mo-Bio, so we recommend calling Mo-Bio's technical support, or e-mailing Tim Hopkins at BioSpec ([hop@biospec.com](mailto:hop@biospec.com)) for information about how the beads compare. Bio-Spec advertises that you can buy vials that are pre-loaded with beads. These must be ordered in increments of 45. BioSpec orders can be filled within 24 hours. Please confirm that the beads you select are designed for use with the tubes you purchase. Some of the metal beads are exceptionally hard and will crack plastic vials. Also, BioSpec has instructions for using the BeadBeater on its website if you would like to further familiarize yourself with this equipment.

There is a fume hood available if you must use to chloroform or phenol in your extraction protocol. There will be solid and liquid organic waste containers available in the hood.

*BioSpec Bead Selection Guidelines:*

<b>Material</b>	<b>Suggested bead</b>
Bacteria	0.1mm diameter glass beads
Yeast/Fungi	0.5mm diameter glass beads
Tissue	1.0mm diameter glass or zirconia/silica beads
Skin/Soft plant material	2.0mm diameter zirconia beads

*Mo-Bio Bead Chart:*

**Bead Application Chart**

Sample Type		Garnet 0.70 mm*	Garnet 0.15mm*	Carbide 0.25 mm*	Ceramic 2.8 mm	Ceramic 1.4 mm	Metal 2.38 mm	Glass 0.5 mm	Glass 0.1 mm
Tissue	Tissue	V			V, B		V, B		
	Skin	V			V, B		V, B		
	Hair	V					V, B		
	Nail	V					V, B		
	Bone				V, B		V, B		
	Lung	V			V, B				
	Muscle				V, B		V, B		
	Liver	V			V, B				
	Brain	V				V, B			
	Heart				V, B		V, B		
	Kidney	V				V, B			
	Gonad	V				V, B			
	Insect/ Fly				V, B		V, B		
Insect/ Tick				V, B		V, B			
Microbial	Bacteria		V, B				V, B	V, B	
	Yeast		V, B			V, B	V, B	V, B	
	Gram +		V, B				V, B	V, B	
	Gram -		V, B				V, B	V, B	
	Fungi / Mold		V, B				V, B	V, B	
	Spores		V, B				V, B	V, B	
Plant	Soft Plant Tissue	V					V, B		
	Tough Plant Tissue				B		V, B		
	Seeds				B		V, B		
	Stems				B		V, B		
	Corn						V, B		
	Nuts						V, B		
	Rice						V, B		
	Wheat	V					V, B		
	Leaves	V			B		V, B		
	Arabidopsis	V			B		V, B		
Soil	Soil	V				V, B			V, B
	Coral	V					V, B		V, B
	Marine Sediments	V							V, B
	Sludge	V							V, B
Fecal	Fecal/Stool	V				V, B			
	Scat	V				V, B	V, B		
RNA	Tissue RNA				B		B		
	Microbial RNA			V					
	Plant RNA				B		B		
	Soil RNA			V					

Recommended Methods: (V) Vortex and vortex adapter; (B) Bead beating instrument; V, B = Either method

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## DNA QUANTIFICATION

### Equipment at HBS:

• **Eppendorf Biophotometer Plus**-This piece of equipment easily and reliably quantifies DNA concentrations of your samples. It requires that you load 50uL of a sample into a disposable plastic cuvette. The machine will read out the concentration. Cuvettes are expensive and available at HBS (but call to check that we still have stock—if not, you will need to bring these), but we recommend the following protocol for quantification in order to use as few cuvettes as possible and save as much of your sample as possible. You can order cuvettes through Eppendorf or secondary carrier (Fisher, etc.) The item is Eppendorf UVette® part number 952010051 or 952010069.

### **Sample quantification protocol:**

1. Make a 1:10 dilution of your sample with purified water
  2. Load 50ul of the diluted sample into the cuvette
  3. Multiply the resulting concentration by 10x to get the concentration of the undiluted sample in your tube.
  4. Rinse cuvette and reuse it, disposing of the diluted sample that you tested.
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## PCR

### Equipment available at HBS:

- **Eppendorf centrifuge 5430** - spins 1.5ml tubes and or 96-well plates, 0.2ml tubes or strips
- **Eppendorf Vacufuge** - spins/dries 1.5ml tubes or 96-well plates, 0.2ml tubes or strips
- **Two Eppendorf Mastercycler pro Gradient Thermal Cyclers**
- **Tuttnauer 2540E Autoclave**
- **Minivortexers and personal centrifuges** for tubes or strips
- **Eppendorf Research Plus1000, 200, 20 and 2uL pipettors**
- **Barnstead EASYpure II uv/uf water filtration system** - provides type 1 water appropriate for all DNA work.
- **Refrigerator, -20 freezer** (2 ultralow -80 degree freezers available in the Coker Lab)
- **Tube racks - 0.2 and 1.5ml**
- **Dry bath incubator** - heats 1.5ml tubes
- **Ice buckets**
- **Flake ice**
- **Glassware**

### Supplies that users must bring:

1. Gloves
2. PCR and microfuge tubes or plates
3. Tips (filter tips recommended)
4. Lab tape and sharpies
5. Purified water for PCR (if desired)

6. All PCR reagents (primers, polymerase, buffers, etc)

**Notes on equipment:**

**Thermocyclers**

The thermocyclers are both gradient cyclers that use 0.2ml tubes, strips or plates. They are backed up with an APC backup battery and backup generator so that if there is a power failure the programs will not be affected. Users can use the guest account (unprotected) or create their own password-protected account with their own personal programs. Once you have created your user account, simply make program name then go to “edit program.” To save program, hit “next” arrows, then save and exit. Creating the program parameters is fairly intuitive, and instructions are available with the unit.

**Centrifuge**

The centrifuge has one rotor that spins 1.5ml tubes and another rotor that spins 96 well plates, strips or 0.2ml tubes. The plate rotor requires you to use the plastic adapter that will support the plates, strips or tubes. You can change the rotor with the supplied allen wrench (stored in the plastic Eppendorf wizard). Simply loosen the bolt and lift the rotor up, place the other rotor on the spindle, and tighten. To communicate to the centrifuge which rotor you are using, use your hand to spin the rotor counterclockwise and within 15 seconds the correct rotor should register on the screen. Be sure to use lids, as they will squeal.

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## VISUALIZATION

**Equipment available at HBS:**

- **Power packs** - available to run 8 gels simultaneously
- **Gel rigs** - small, med, large and extra large
- **Balance and measuring trays**
- **Microwave**
- **Ethidium Bromide solution**
- **Transilluminator**
- **Photodoc-IT 50 imaging system with camera, lcd screen and printer**

**Supplies that users must bring:**

1. TAE/TBE
2. Agarose
3. Ladders
4. Loading buffer
5. Tips
6. Gloves

**Notes on gels:**

Ethidium bromide solution is made from a 1% solution diluted to a 1000x concentration. You will need one uL of EtBr solution for each ml of gel solution, and the small Owl rigs require 50-60ml gels. Dispose of buffer that has been exposed to EtBr gels in glass

bottles under sink. You can minimize liquid disposal by reusing buffer for multiple gel runs. Dispose of gels in white plastic bucket and contaminated buffers in glass container.

**Note: Backup Power**

The lab is protected by a backup generator that will power the freezers and fridge in case of power outages. Thermocyclers and Vacufuge are plugged into a UPS backup APC battery that will keep programs going until the generator goes on or the power is restored, providing continuous power (no interruption in programs).

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**IF YOU WOULD LIKE TO VISIT US AT THE TREE HOUSE:**

**Reserve the laboratory:** Please contact the HBS office, 828-526-2602, to let us know when you plan to use the molecular lab and the nature and duration of your intended work here. We want to be sure that the lab suits the needs of all users and that all planned activities will be compatible! In addition to the workbenches in the lab, we have four desks available on the molecular lab floor for \$50/week, so let us know if you would like to reserve any additional workspace. Users that are already paying a bench fee in the Coker lab or paying for desk space in the Tree House may use the molecular lab for an additional \$20/day or \$100/week. If only using the molecular lab, fees are \$25/day or \$150 per week for full access to the laboratory space, equipment and glassware.

**Bring your own supplies:**

While researchers must provide all of their own reagents, disposables, and general lab supplies (gloves, tips, etc.), some disposables and reagents are available for an additional fee, if needed. We cannot guarantee that we will have exactly what you need, so please take the time to be sure you have everything to complete your experiments!

