Prather Lab Chore Protocols

Updated December 17, 2008

Chores in the Prather lab are shared tasks performed on a rotating basis. You are expected to pay attention to the lab's chore sheet and to perform the various lab chores in a timely manner when it's your turn. This document provides instructions for performing all of the lab chores.

Disposing of Aspirator Waste

Aspirator waste should be disinfected and disposed of when the aspirator flask contains about 900-1000 mL of liquid.

1. Remove the plug from the top of the aspirator and set it aside. Disconnect the aspirator flask from any tubing. Move the flask to a location where you can easily work with it.

2. Disinfect the aspirator waste by adding pure bleach (found in the cabinets underneath the lab's main sink) to a final dilution of 1:10 directly into the aspirator flask. Swirl the flask to mix. Let the flask sit for 20-30 minutes. The bleach should be added even if the flask contents are clear.

3. Pour the disinfected contents of the flask slowly down the lab's main sink and flush with plenty of water.

4. Add about 100 mL of bleach to the empty flask.

5. Reconnect the flask to the vacuum pump and re-plug the flask.

Autoclaving Biological Waste

Waste from the white biohazard waste bins must be autoclaved before disposal. Biological waste must be autoclaved for extended periods of time (~90-180 minutes) to ensure its sterility. Because treating biological waste requires extensive use of the autoclave (which is shared by several labs), it is more polite to only autoclave waste in the evenings or on weekends.

1. Close the large, clear biological waste bags with cable ties. It's a good idea to fill up any less-than-full bags with any full, small, clear biowaste bags from the benches.

2. Fill out and tie an "Autoclaved Biological Waste" white tag around the neck of the bag.

3. Take the biological waste bags to the autoclave room (56-415).

4. Fill out the "autoclave log sheet" in the autoclave room (the one that asks for the autoclaved waste bag tag numbers).

5. Set the autoclave to dry and make sure the temperature is set to 121^{o}C. Set the sterilization and drying times to the following:

Number of Bags	Sterilization Time (min)	Drying Time (min)
1	90	20
2	105	20
3	120	20
4	130	20
5	140	20
6	145	20
7+	150	20

6. Let the autoclave run. Be sure to let the autoclave depressurize before removing your trash bags. Note that it is ok to let the autoclave run overnight, as long as you pick up your waste the next day.

7. Remove the waste bags from the autoclave and discard them in the lab's normal (not biological) trash bins.

Note: Call EHS at 2-3477 to request additional autoclave bag tags (white).

Disposing of Biological Sharps Bins

The biological sharps bins are picked up and disposed of by EHS every Thursday.

1. Push any pipets or other waste down into the container, such that nothing is sticking out above the rim of the bin.

2. Close the bin by pulling the bin's cover all the way over the top of the bin.

3. Use two cable ties to seal the bin's cover. Holes for cable ties are found on one side of the cover.

4. Send an email to biosharps@mit.edu no later than Tuesday evening with the following information:

    - Your Name
    - Your Contact Information (Phone and Email)

- Lab's Building and Room Number (66-425)
- Number of Biosharps Bins to be Collected

If you send them an email on Wednesday there is still a good chance they will pick up the bins, but not always.

5. On Wednesday evening place the filled, sealed biosharps bin(s) out in the hallway.

6. On Thursday, EHS will come by to remove the filled bin(s). They will only come by if you sent them an email on Tuesday (or earlier). When they remove the filled bins they will replace them with empty ones. EHS can come by anytime between 7:30am and 5:00pm to pick up the filled bins.

7. Move the new empty bins into the lab and place them in the lab appropriately.

Decontaminating the Ethidium Bromide (EtBr) Waste

The EtBr waste should be decontaminated when the 5L EtBr waste container (found at the gel station in the lab) is 90-95% full. When treating the EtBr waste, wear the appropriate personal protective equipment (gloves, safety glasses, lab coat) and be very careful not to get any of the waste on your skin or to splash any of it on the benchtop. EtBr, as a DNA-binding molecule, is a strong mutagen. Be sure to change gloves if they get wet with EtBr waste and before going back to other labwork. Try not to spread EtBr around the lab.

Currently, the lab uses the Extractor EtBr System from Whatman. From the company's website:

The Extractor™ EtBr System is a one-step filtration funnel device for the rapid removal of ethidium bromide from gel-staining solutions.

This disposable unit contains an activated carbon matrix, which removes > 99% of ethidium bromide from electrophoretic buffer quickly and easily. Each device can decontaminate up to 10 liters of gel-staining solution. After filtration, the decontaminated solution can be safely poured down the laboratory drain.

The extractor funnel device fits most standard laboratory flasks and bottles (neck size 33 to 45 mm), and the unit includes a cap for storage between uses. the polypropylene housing is chemically resistant to organics. also included in the package are glass fiber prefilters, which remove gel pieces and other debris to avoid premature clogging of the carbon filter.

1. Obtain an EtBr filtration funnel and two white prefilter disks from the cabinets underneath the sink near the gel station. If there is a partially-used EtBr filtration funnel be sure to use that one. Also find a 1L Erlenmeyer flask. Place these items near the vacuum pump, or bring the pump over the gel station on a cart.

2. Disconnect the aspirator from the vacuum pump. Using vacuum tubing (not the clear tubing), connect the vacuum pump to the EtBr filtration funnel. Place the funnel on top of the 1L flask. Place one of the prefilter disks on top of the black, activated carbon inside the filtration funnel. The orientation of the filter disk (face up or face down) does not matter.

3. Fill the EtBr filtration funnel with EtBr waste and turn on the vacuum pump. You may need to pipet the first ~500 mL of waste from the 5L waste container to avoid spilling if the container is too full to pour.

4. Allow the vacuum to draw the liquid through the filter. Repeat step 3 until the 1L flask is full.

5. Flush the contents of the 1L flask (the decontaminated liquid) down the sink with plenty of water.

6. Repeat steps 3-5 until all of the EtBr waste is decontaminated and disposed of. You will notice that the more liquid you treat, the slower the liquid will flow through the filter.
    - If the liquid flow rate becomes very slow, replace the prefilter. Used prefilters should be disposed of in the black chemical waste bucket.
    - If changing the prefilter does not improve the filtration rate, replace the entire EtBr filtration funnel. Dispose of the used funnel in the chemical waste bucket.

7. Place the empty 5L EtBr waste container back at the gel station. Disconnect the vacuum tubing from the funnel and reconnect the aspirator.

8. On the side of the EtBr filtration funnel, check off the volume of waste that you treated (typically 5L for a full waste container). Each filtration unit has the capacity to decontaminate 10L of waste. If the unit you are using has reached 10L, dispose of it in the chemical waste bucket.