Preparation of wet sediment samples for freeze drying (can be part of ^{210}Pb analysis)

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1. Send a lab e-mail stating your intent to use the freeze dryer at a particular time.

2. Weigh all of your bags of sediment and record the mass prior to any kind of subsampling (weight of whirl-pak + sediment). Be sure to dry the outside of the bags first. To get the weight of wet sediment you will need to subtract the weight of the empty whirl-pak bag. Weigh ~20 empty whirl–pak bags of the same type your sediment is in and take the average weight.

3. Decide what intervals in the core you will be dating. Typically, you will prepare ~15 samples for the gamma counter (this can vary). Remember that you can interpolate dates so you need not process all samples as this will be very expensive. As your samples go through the gamma counter you should check whether you have reached background ^{210}Pb levels so that you will not be running numerous samples needlessly.

4. Set up a spreadsheet to record your data and decide what intervals you will be dating. These data are important so it is best you transfer your data to a spreadsheet and save the file soon after you weigh your sediments. *An example of how you can set up your spreadsheet is given below*.

Northern Ontario Lakes: **North Raft Lake- Maxi-Glew (labelled KB-1):**

Green = Chla
Yellow = Pb-210

				FREEZE-DRIED SEDIMENTS								
Interval cm		Bag + sed wet wt (g)	Wet sed (g) Entire sample	Vial wt(g) incl. lid	Vial + Wet Sed incl. lid	Wet sed (g)	Vial - Dry sed incl. lid	Dry sed (g) in vial	% water Es	Dried sed (g) in gamma tube	Tube height (mm)	Wet Sed Diatoms (g)
0		40.709	37.1590	6.21	23.388	17.1780	7.9256	1.7156	90.0128	0.8747		
0.25		12.856	9.3060	6.158	14.453	8.2950	7.0662	0.9082	89.0512	0.6953		
0.5		14.056	10.5060	6.137	14.383	8.2460	7.1463	1.0093	87.7601	0.7066		
0.75		16.395	12.8450	6.173	16.086	9.9130	7.5156	1.3426	86.4562	0.6341		

5. Label plastic scintillation vials and caps with your name, lake, and interval depth. This information should also be etched into the plastic vials as the marker tends to fade or rub off with handling and time. Many of our sediment samples are now stored in the freeze-dried state and if these sediments are to be used for future analyses (e.g. additional indicators) these samples will be useless if this information is missing. *Cautionary note*: Recently, PEARL was sent samples in plastic scintillation vials that were freeze-dried here at PEARL where the labelling had completely rubbed off leaving very little indication of which sample was within.

6. For each interval:

i) Weigh the empty scintillation vials with out caps- record the mass. As soon as freeze dryer cycle is complete tighten caps and place capped vials into desiccators.

ii) Mix your sediment sample well and move the sediment towards one side of the whirl-pak bag (this makes it a bit easier to scoop sediment). Applying clean techniques, use a metal (or plastic) spatula to scoop the sediment into the scintillation vial. Fill the scintillation vial ½ to ¾ of the way full. For more watery, top intervals you may find it easier to use disposable polyethylene transfer pipettes with the narrow tip snipped off.

iii) Record the new mass of the scintillation vial with cap, plus wet sediment.

iv) Once all sediment is weighed for all intervals you wish to freeze-dry, loosen caps 1 turn and place them in the freeze-dryer tray.

v) Ideally, you would want to center your vials in the middle of the tray so that the vials will be in the premium location (centered over the warmest part of the shelf) within the freeze dryer.

If freeze-drying entire Whirl-Pak bags, it is best to use the Labconco freeze dryer in the teaching lab (3rd floor) or the Thermo Savant ModulyoD. Seek training on all these units if you have not used it before and see instructions on the PEARL Internal webpage.

For the Virtis Advantage freeze dryer in our wet lab (Room 4312), arrange bags as seen in the photo below. It is best to run the Virtis Advantage in manual mode (see instructions on website and above freeze-dryer). For this method, the bags are placed in a freezer for at least 3 hours. Before placing them in the freezer, make sure the bags are flat and the sediment is concentrated toward the bottom of each bag (if you freeze them folded over, you will not be able to unfold them when placing them into freeze-dryer).

NOTE: Before you place the bags into the **freezer**, make sure that you roll over the top of the Whirl Pak bag about 3 times to ensure that your wet sediment does not ooze out of the bags before they freeze.

HOWEVER, when you are ready to place these bags into the **freeze-dryer**, make sure that you unroll each bag fully and cover over the tops with a Kimwipe secured with the wire bag closures. Make sure the bag is fully unrolled before placing the folded Kimwipe over the top but do not expand or widen out the mouth of the bag. Unrolling the bags will allow pressure to escape and ensure that the bags will lie flat on the tray surface (If not unrolled, they will puff out and stand up straight with minimal contact to the tray bottom- and the sediment will not freeze dry). Contact of the bags with the tray surface is essential to conduct heat between the samples and the shelf. Securing a Kimwipe over the unrolled bag will avoid cross-contamination. Stacking bags as in the photo below allows this contact. To check, lift the tray and look at the bottom to ensure that all bags have a nice contact with the tray bottom. Don't cram so many bags on the tray that they can't have at least some lateral contact with the tray. The bags should not be stacked two or three deep or just have enough room to stand vertically. You must work quickly or with the bags still sitting in the freezer to avoid thawing of the sample. There are freezers beside both Freeze driers.

When using the Labconco freeze dryer in the teaching lab, you simply put the samples in the manifold and spread out the bags as much as possible. The Labconco can hold about twice as many bags than the Virtis. However, the more bags the longer the time needed to complete the process.

The freeze dryer will typically be full when you place ~50 plastic vials on the tray. Keep in mind that the more samples you include, and/or the more sediment is measured in each vial, and the wetter the sediment will result in longer freeze-drying time and may mean that you will have to run the samples twice to achieve satisfactorily freeze dried samples. You may find that one run will be sufficient, particularly if you have sediment from deeper parts of the core (drier) or if a small amount of sediment is in the vial, or you do not have many vials. Each run of the freeze dryer takes about 22 hours (see instructions pasted above freeze-dryer). You may also consider placing your samples into a freezer at least 3h prior to placing them in the freeze-dryer. If you do this, you would opt to run the samples manually as this will skip the first stage of the freeze-dryer (freezing stage). [Note – Inserted by BFC 22 Sept. 2014 – **please talk to your supervisor before choosing to freeze your samples using the VIRTIS freeze drier, or in a freezer.** There may be differences related to crystal formation if a sample is frozen slowly (freezer) or flash frozen at -50 degrees C. Briefly stated, the slower the freeze, the larger the crystals. The choice of method will depend on the intended use of the freeze dried material. It is essential to state how you froze your sample in your methods, and to ensure that samples within a core are treated in the same manner.] The freezing stage of the freeze-dryer takes approx. 120 minutes. The vacuum pump, shelf heat and condenser will then come on. The shelf temperature after freezing is ~-50 °C and this temperature will need to be raised to the temperature set by the recipe (35 ºC). Therefore, the manual method (i.e. using a deep freezer) is about 2.5 hours shorter than the recipe method. Regardless of which option you choose, you may need to run your samples 2X depending on the number, amount and wetness of your samples. But often one run does it. Again, see the step by step instructions posted above the freeze dryer.

7. Using the freeze dryer:

NOTE: there are two approaches that can be used. One is following the "recipe" (#3) programmed into the freeze-dryer. The second is running the freeze-dryer "manually". Both sets of instructions are posted beside the freeze dryer.

i)<u>If using the "recipe" method:</u> Read and follow the directions on the sheet posted near the freeze dryer. We no longer use the probes as we have in the past so do not worry about these. Make sure the drip tray is in place underneath the condenser coils at the bottom of the freeze dryer.

ii) If you are using a recipe (that is if you are putting your samples through an initial, automatic freezing stage) you should choose recipe #3 (shelf temperature of 35ºC). Recipe #2 has a shelf temperature of 25C and takes much longer. This will appear on the main menu of the screen.

iii) After approx. 22 hrs, follow the shutdown directions posted on the freeze dryer. Remove the tray containing the vials and immediately check the temperature of the outer and wetter vials (your upper lip is your most sensitive test for non-toxic samples) to determine if there is still moisture (ice) in your sediment. Work quickly to prevent sample thawing. If the vials are at all cold, the freeze drying is not complete. If all the samples are warm like the shelf, the process is likely complete. You can also check if the samples are dry by removing the Kimwipe from a few vials and using a spatula to stir the sediment look for ice or clumping (or put cap on and shake to hear any rattling). If you feel or hear any hard lumps, this is probably frozen sediment, which means the sample still contains water (don't get fooled by hard packed sediment or pebbles). If ice remains, you must continue freeze drying. Depending on the amount of ice buildup on the condenser coils, you can either immediately restart the freeze dryer manually, or defrost the coils before continuing the process. If a defrost of the coil is needed, place your samples in a deep freezer while the condenser coil thaws. When the coil is clean and dry, return the samples to the shelf and restart the freeze dryer manually – see instructions for "Manual" method. Allow the freeze dryer to run for several hours before checking the sample temperature.

iv) When samples are dry, remove them and their corresponding caps from the freeze-dryer and immediately cap them and place them into a desiccator.



The desiccators are filled with blue crystals (Drierite) that turn pink when the material has expired (i.e. ensure that it is blue not pink- if pink, you will need to replace with blue crystals). The Drierite absorbs any moisture thereby keeping the inside of the glass container (desiccator) dry. Once the Drierite has absorbed too much moisture, it turns pink and you will need to regenerate the crystals.* Placing your samples immediately into the desiccators takes very little effort and is simply a little extra insurance that you will get accurate calculations for % water and more importantly, weights of the dried sediment placed into the gamma tubes. This is particularly important when prepping sediment for ^{210}Pb procedures – please read methods for prepping sediments for dating.

- *[Note: *although time consuming, DRIERITE can be reused by regenerating it after it is exhausted. This can be done by removing it from the desiccator and heating it in an oven. The granules should be spread in layers one granule deep and heated for 1 hour at 210° C or 425° F. The regenerated material should be placed in the original glass or metal container and sealed while hot.* For more information - http://www.drierite.com/default.cfm].

NOTE:

Once the cycle is complete, there will be ice on the condenser coils in the freeze drier.

After each cycle, you must thaw out the condenser coils. This is particularly important if you plan to undertake another run right away. If this is the case, there is a small fan near the freeze drier. Place the fan in front of the freeze dryer, leave the door open and turn the fan on high. The condenser coils will thaw out in approximately 30-40 minutes. After it has thawed, remove the drip tray from the bottom of the freeze drier, empty and dry it and the condenser with a paper towel. Replace the drip tray and you are ready for another cycle.