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Preparation of Tissue Samples for DNA Extraction and Copy Number Analysis by iDNA Technologies V.2

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External link: <https://www.idnagenetics.com/>

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Protocol status: Working

We use this protocol and it's working

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Abstract

Preparation of leaf tissue for shipment to **iDNA Genetics** for DNA extraction and copy number analysis.

NOTE: TOBACCO TISSUE IS REGULATED AS DRUGS AND ALCOHOL. iDNA maintains special permits that allow the import of small quantities of tobacco tissue for destructive analysis only. Before sampling tobacco tissue, contact iDNA for up to date required forms and special shipping instructions not contained in this protocol. As of 6/11/2024, iDNA Technologies contacts are michala.woodvine@idnagenetics.com and gelli.christodoulou@idnagenetics.com.

Alternatively for tobacco samples, extract DNA using Qiagen DNeasy Plant Kit and ship per protocols.io protocol "Preparation of DNA Samples for Copy Number Analysis by iDNA Technologies".

For all soybean, cowpea, miscanthus, sorghum, maize, either tissue or DNA can be sent if iDNA has established primers for analyzing copy number. However, iDNA prefers to receive tissue when possible.

Materials

Materials

- Cork Borer Sets with Handles (Fisher, **S50166A**)
- Solid Rubber Stoppers (Fisher, **14-130S**)
- Collection Microtubes (racked, 10 x 96) (Qiagen, **19560**)
- Collection Microtube Caps, (Qiagen, **1051163**)
- Needle
- Parafilm (Fisher, **S37440**)
- Cardboard box

Equipment

- -80C Freezer
- Freeze dryer, Labcono 2.5L FreeZone or equivalent
- Optional: Bel-ArtTM Space Saver Vacuum Desiccators (Fisher, **08-594-16B**)

Get a Quote

- 1 Email iDNA Technologies for a quote. Include the number of samples to be tested, which genes you want copy number analysis on, and which species the samples are from.

Include one sample with a known copy number of 2 as an internal reference standard for each assay (gene).

Note

Minimum of 24 samples is required for analysis. Contact other members of the lab and transformation facility if you do not have the minimum to see if they will be sending samples soon.

As of 6/11/2024, iDNA Technologies contacts are michala.woodvine@idnagenetics.com and gelli.christodoulou@idnagenetics.com.

Note

For required assay (gene), commonly used assays for RIPE include the following:

- VPZ_Bar is called BarPatF3 in iDNA Technologies assay list
- AP3_Bar is called JICBar in iDNA Technologies assay list
- A mix of genes may be possible for certain combinations.
- For genes other than these, email the plasmid or gene oglio sequence to the iDNA team to determine the best assay for your needs.

- 2 When you've received the quote, enter it into the appropriate lab group's [Quartzy](#) (or other purchasing request platform) account.

Enter the following information:

Vendor: i-Dna Biotechnology

Catalog #: xxxxxxxxx

Unit size: 1

Qty: 1

Unit Price: From quote (convert from British pound to US dollar before entering)

Upload File: Attach quote from iDNA

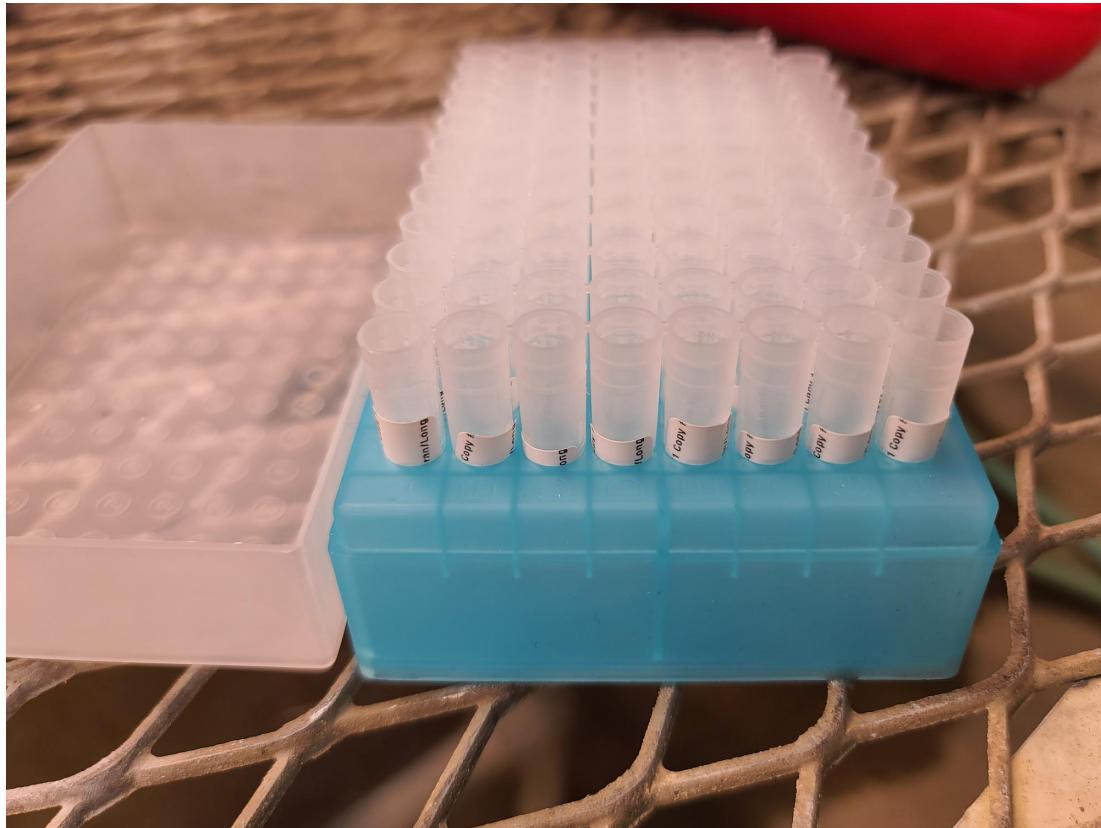
Grant ID: Appropriate CFOP for project

Sample Tissue

- 3 Pre-label the collection tubes.

Note

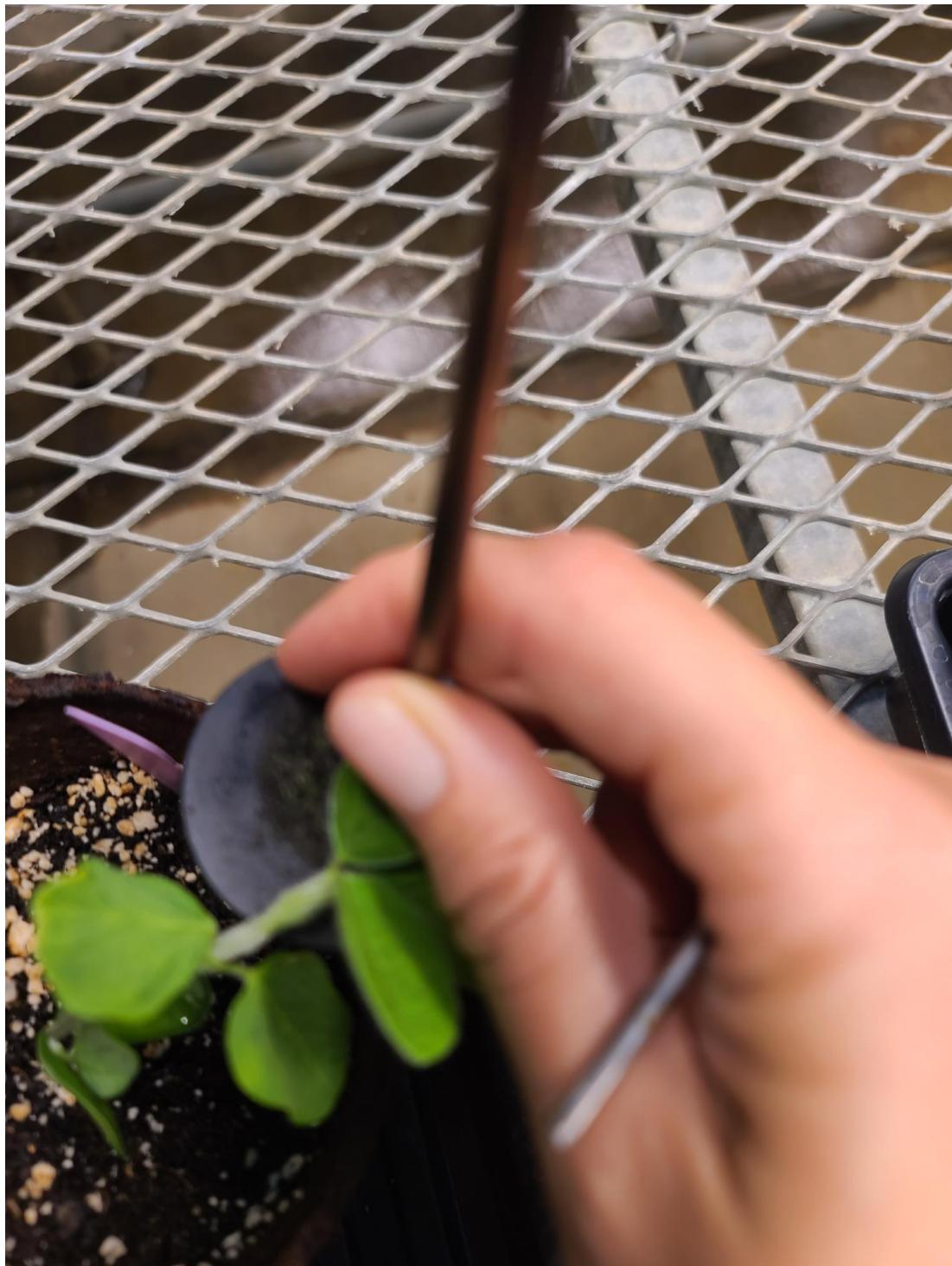
Labelling of tubes is for ease and accuracy of sample collection only. Please be aware that iDNA identifies samples by sample collection box number and well location as provided on the completed sample template, "96 well template_iDNA.xlsx", ONLY. They do not cross-reference the tube labels, the tube locations, and the provided 96 well template.



- 4 Sample three to four #1 Humboldt cork borer leaf punches (4 mm) from the most recently developed leaf by pressing the cork borer through the leaf onto a rubber stopper and using a twisting motion.

Note

Do not sample more than 4 leaf discs. If too much tissue is sampled, it will interfere with DNA extraction.



- 5 Push the leaf discs out into the bottom of its pre-labeled collection microtube.



- 6 Repeat for all desired samples.
- 7 Store collection tubes on ice while sampling. Tubes can be at room temperature for short periods of time (i.e. while you are sampling a strip or box of tubes).

Note

It is not necessary to use dry ice or liquid nitrogen at this time. Regular ice is sufficient.



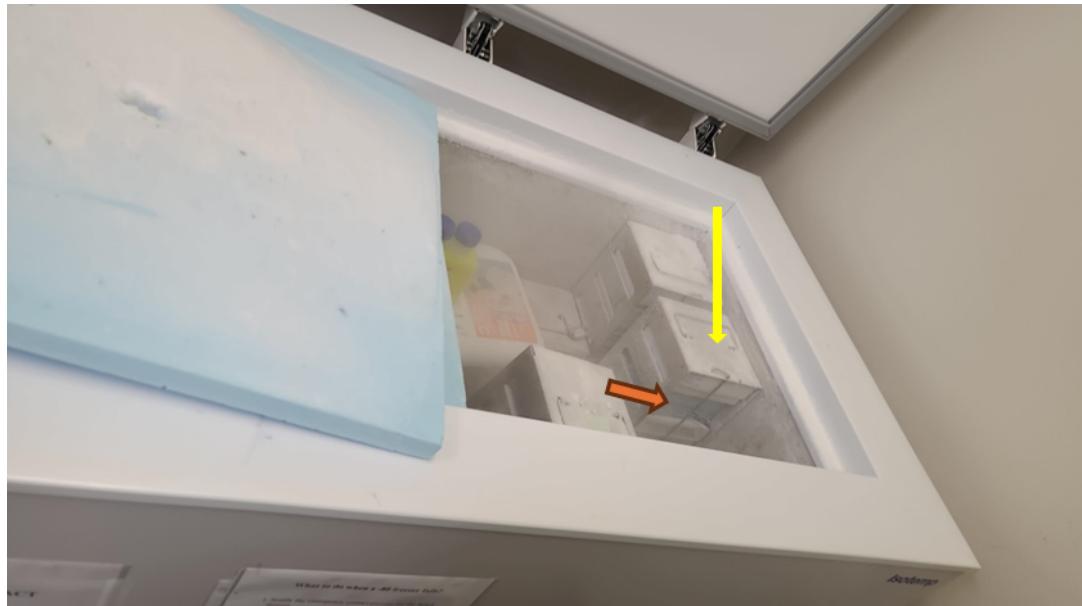
- 8 If sending an internal reference standard, a previously collected sample with a known copy number of 2, add it to the collection tube box from the -80C freezer.

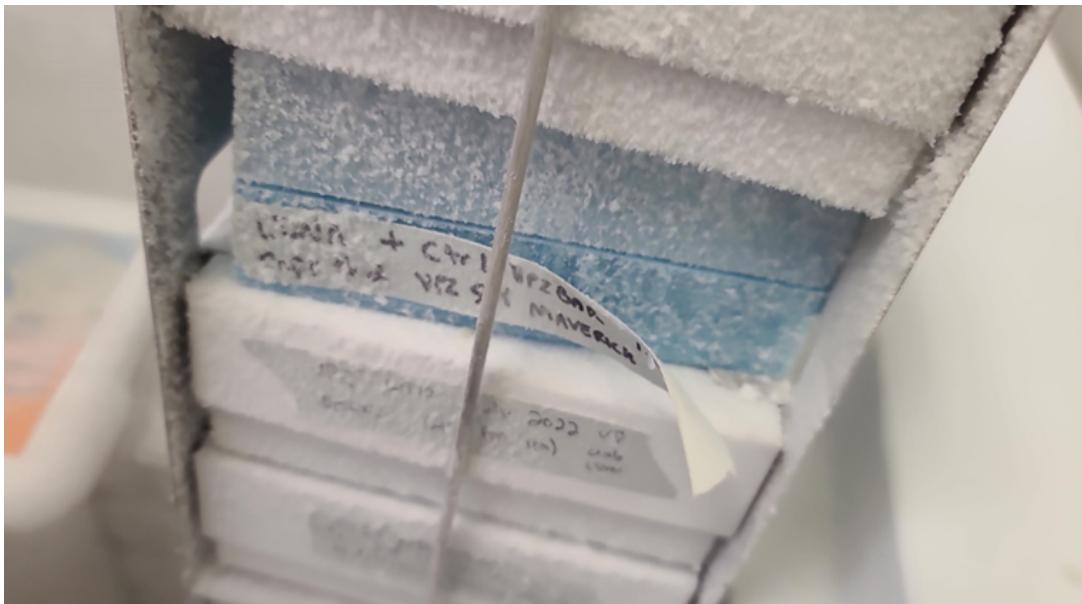
Note

For VPZ_Bar (BarPatF3 in iDNA Technologies assay), known single insert homozygotes (>T5) are prepared and stored in the horizontal freezer in the back hallway of the IGB in room "1312 EBI -80C Freezers".

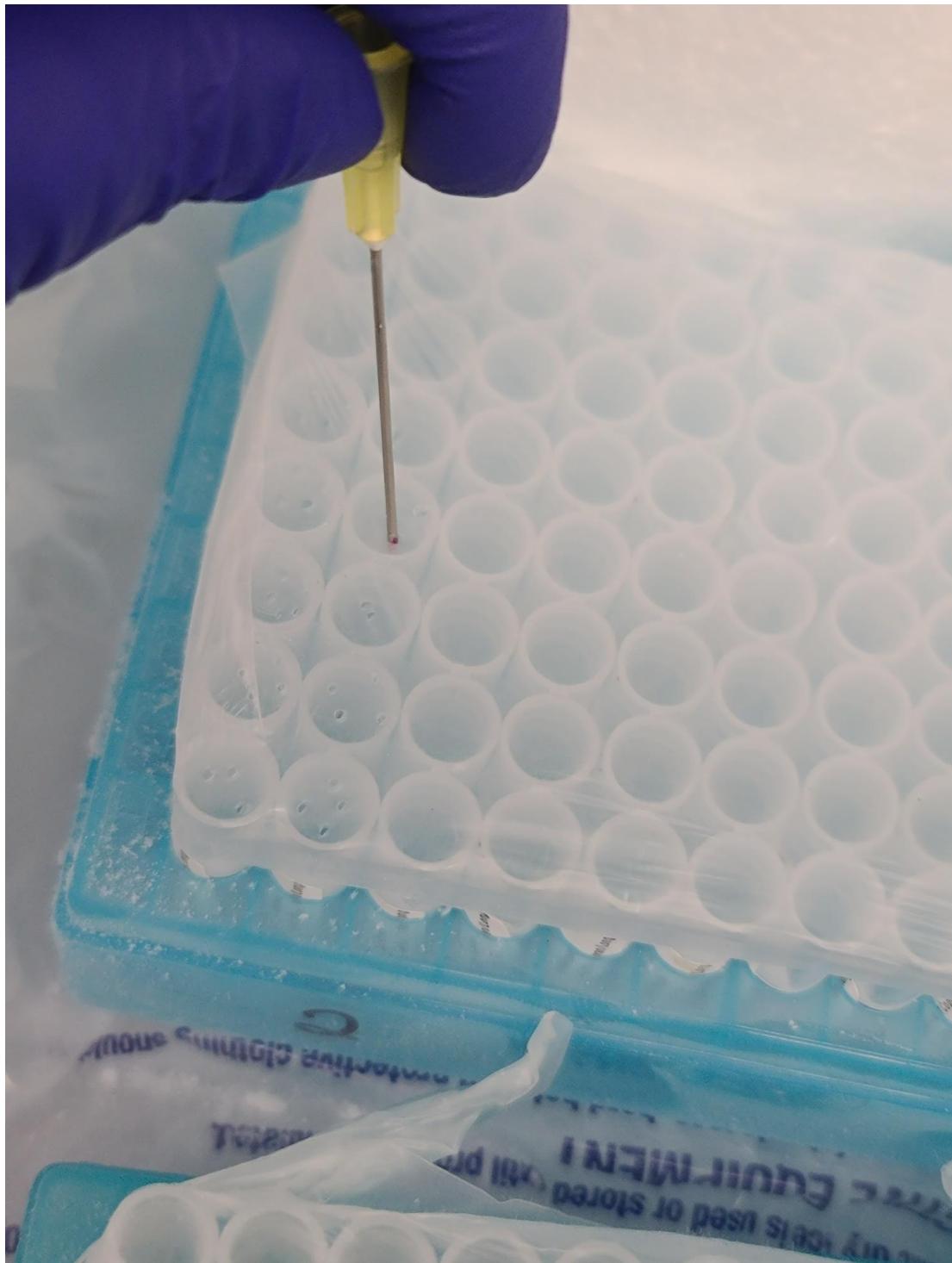


They are in the middle vertical rack on the far left of the freezer in a blue iDNA tube rack box second slot down from the top.





- 9 Stretch a sheet of parafilm across all tubes in a rack.
- 10 Poke pin holes using a needle or 10 ul pipette tip in the parafilm over each tube.



11 Cover the tubes in the rack with their lid.

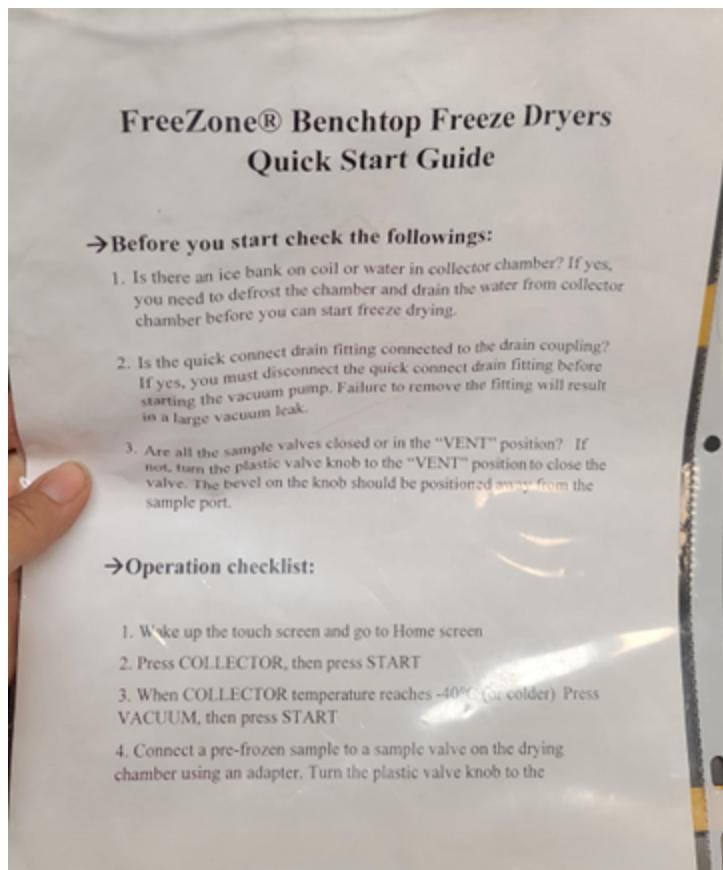
12 Freeze at -80C overnight.

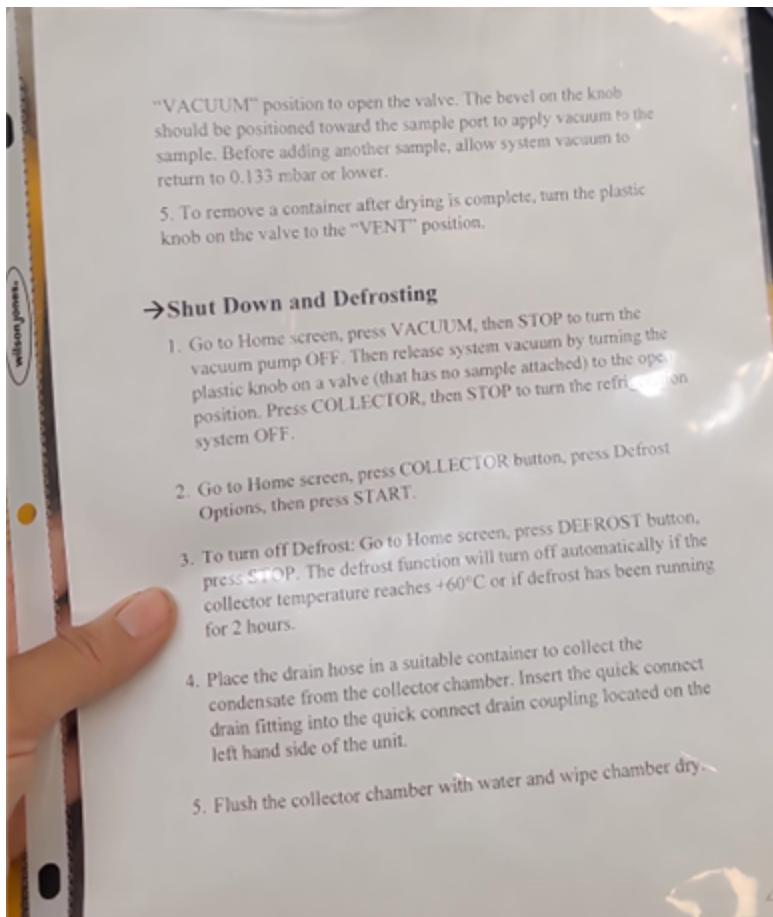
Note

This step is required in preparation for proper freeze drying. A shorter freezing time may be possible but would need to be determined empirically based on the size and quantity of samples.

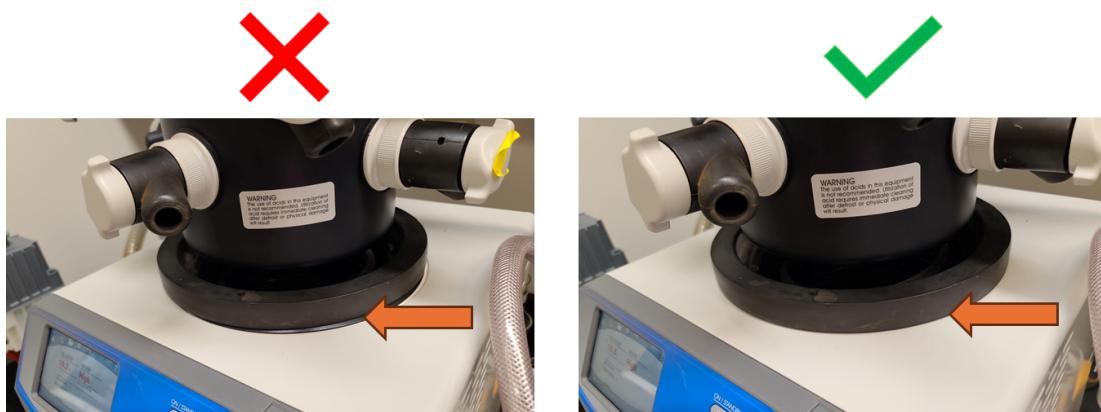
Freeze Dry Samples

- 13 Prepare freeze-dryer by pre-cooling and vacuuming to equilibration based on manufacturer's and lab manager's instructions.
 - 13.1 Review Lab Manager's Quick Start Guide near the instrument.





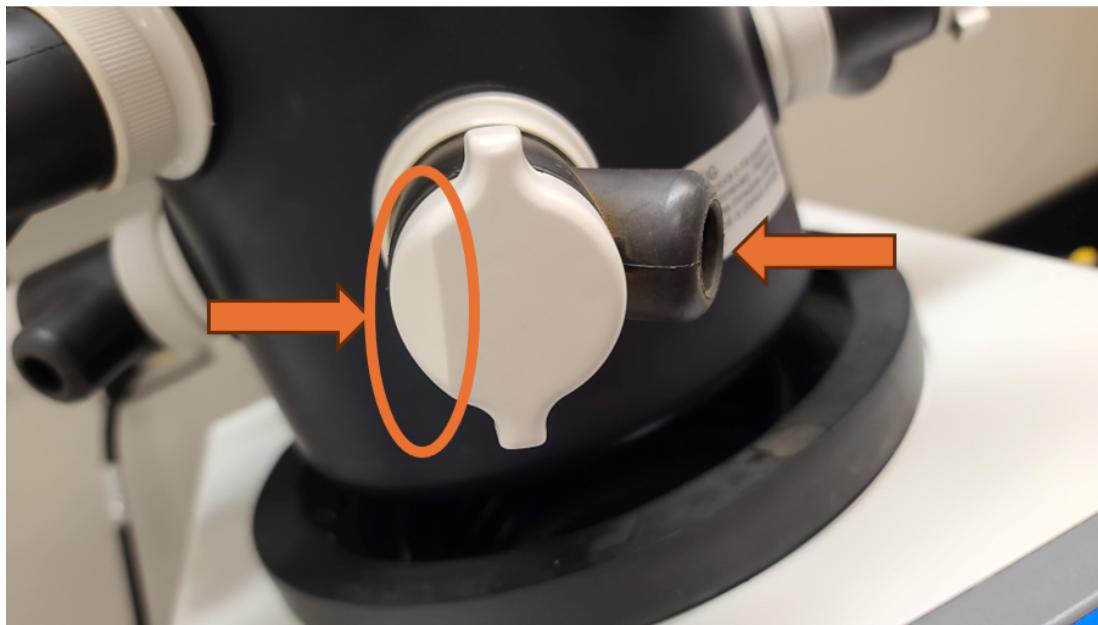
13.2 Ensure that vacuum manifold is squarely lined up with gasket on top of instrument.



13.3 Ensure that all vacuum ports on the manifold are closed. Vacuum ports are closed when the middle of the flat portion of the port handle is pointing in the opposite direction from the

attachment tube.

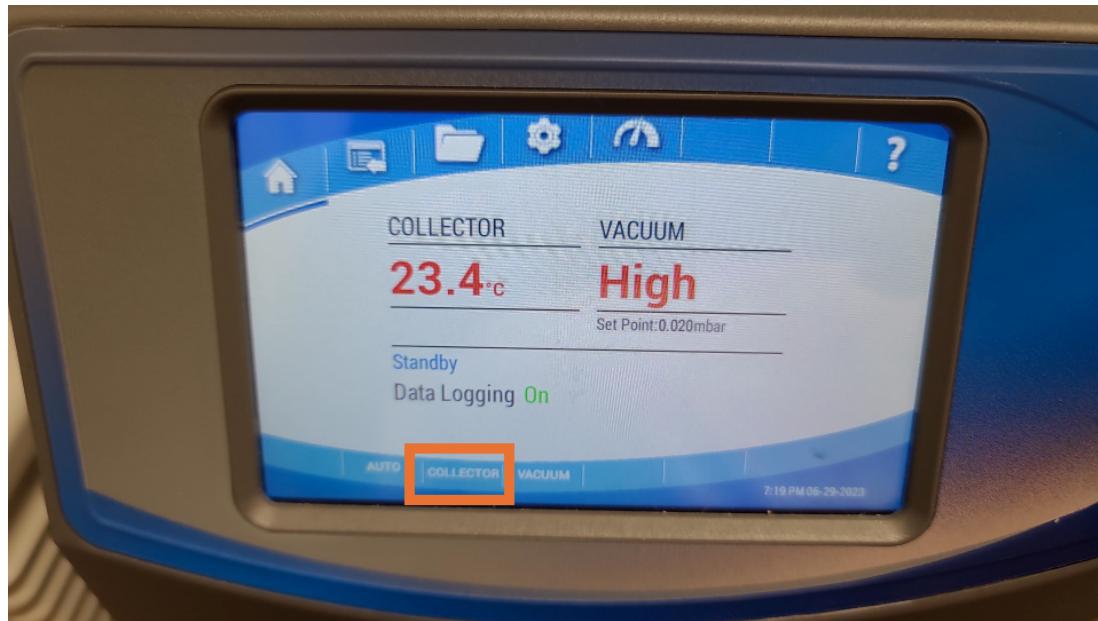
Closed Vacuum Manifold Port



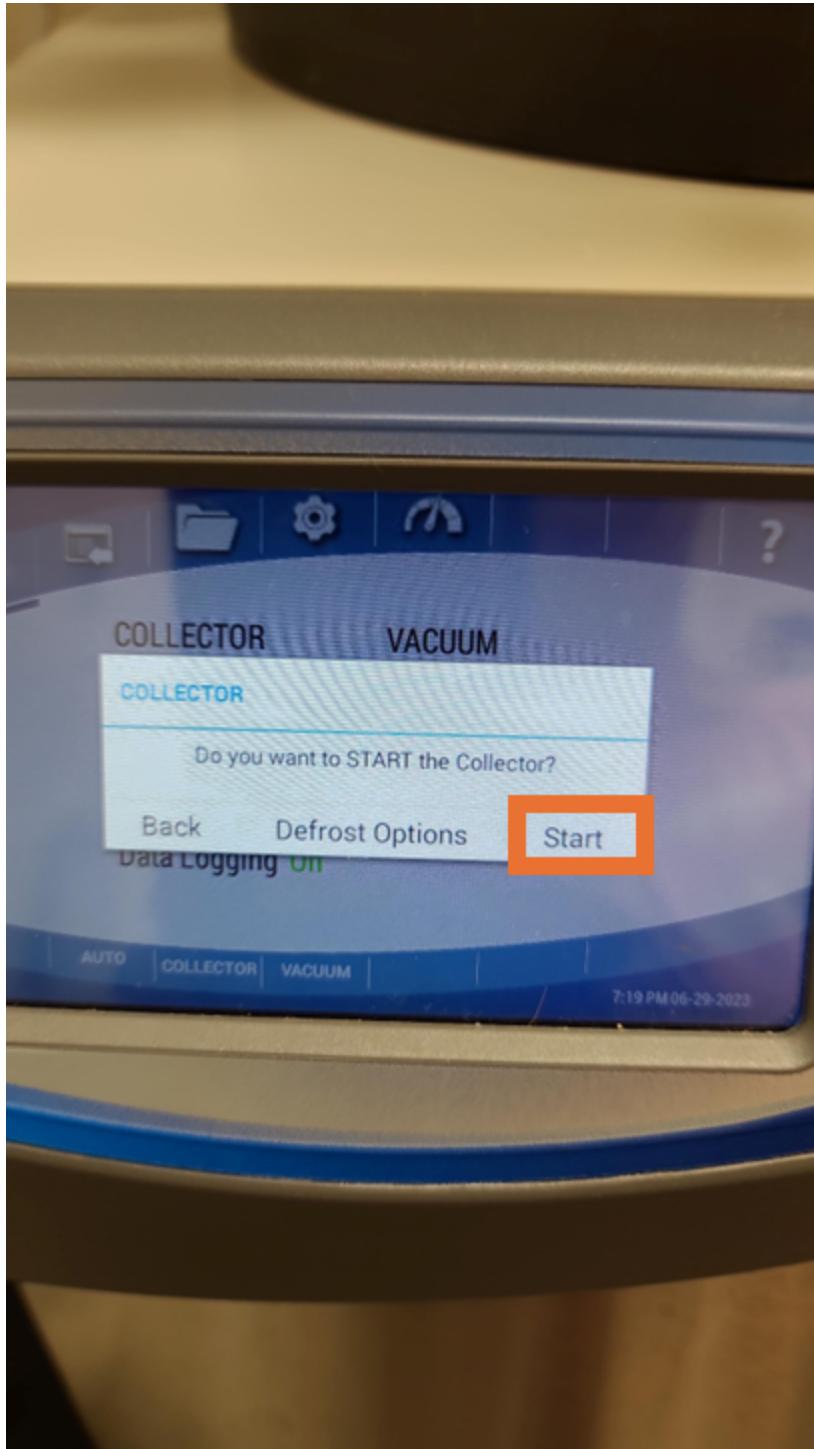
13.4 Press anywhere on the touch screen to wake up the instrument.



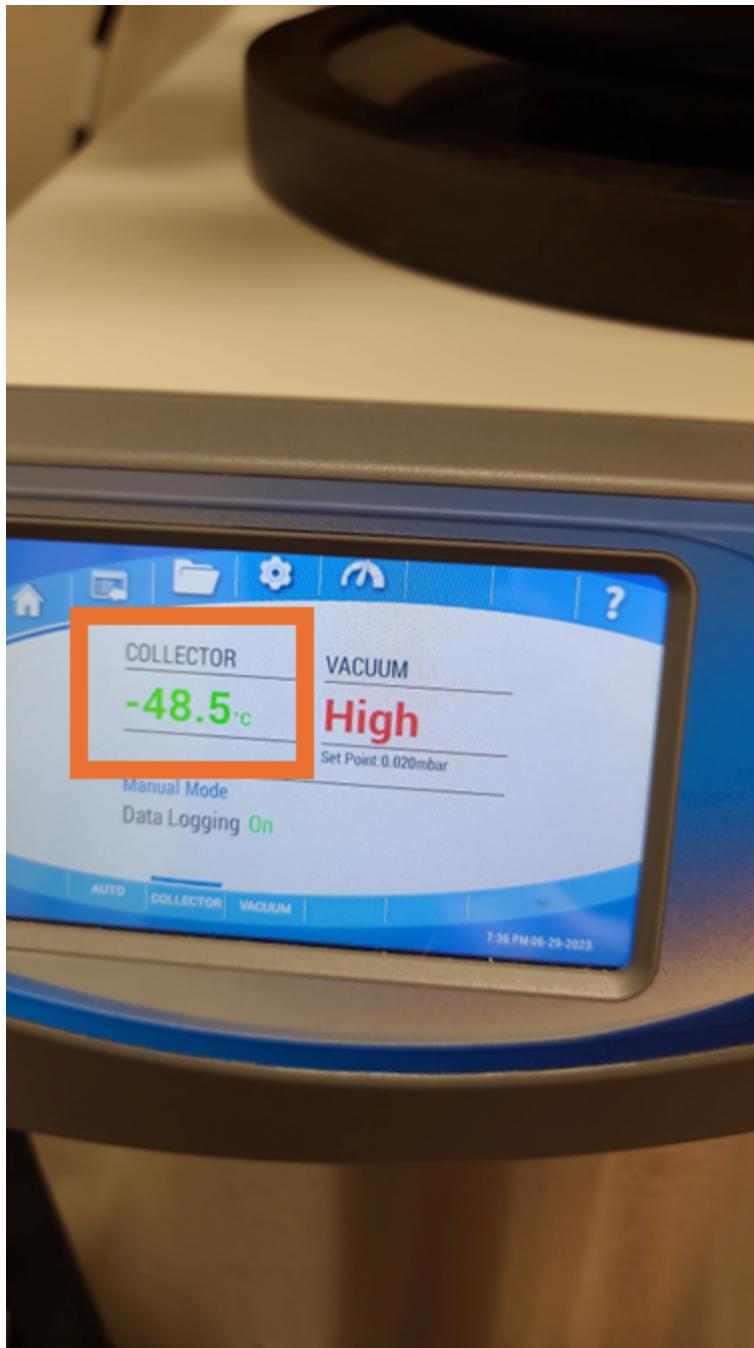
13.5 Use the touch screen to select "Collector" at the bottom left of the screen.



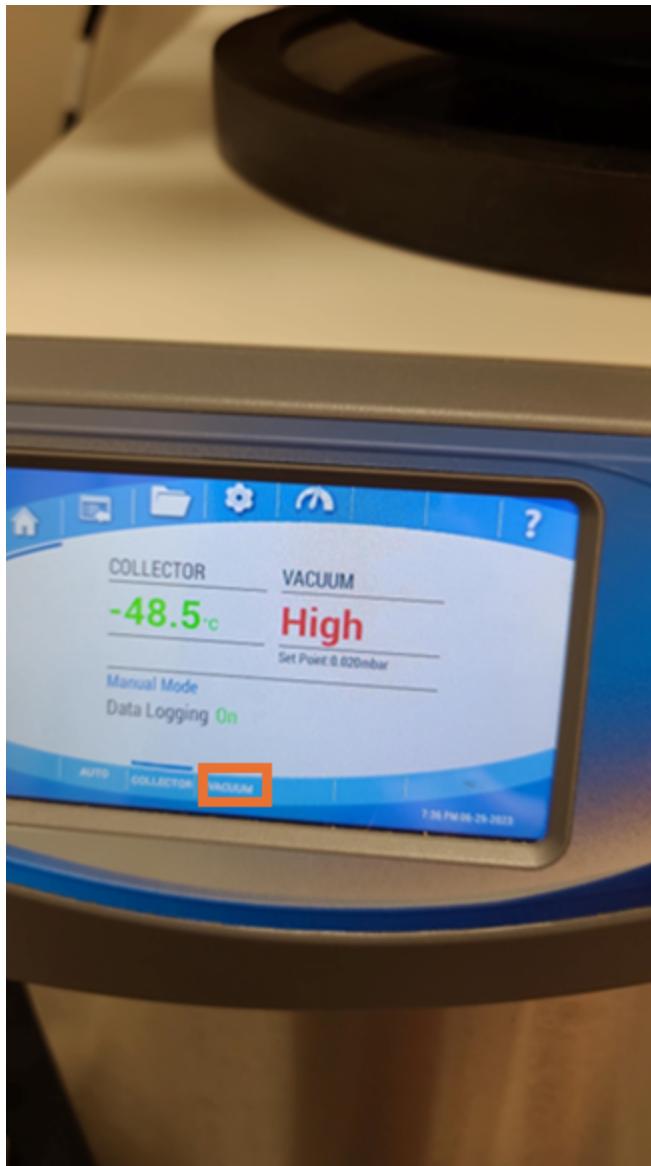
13.6 When prompted, use the touchscreen to press "Start" to start the collector.



- 13.7 Wait for the collector to reach temperatures colder than -40C. The temperature reading on the touchscreen will turn green when the collector has cooled sufficiently.



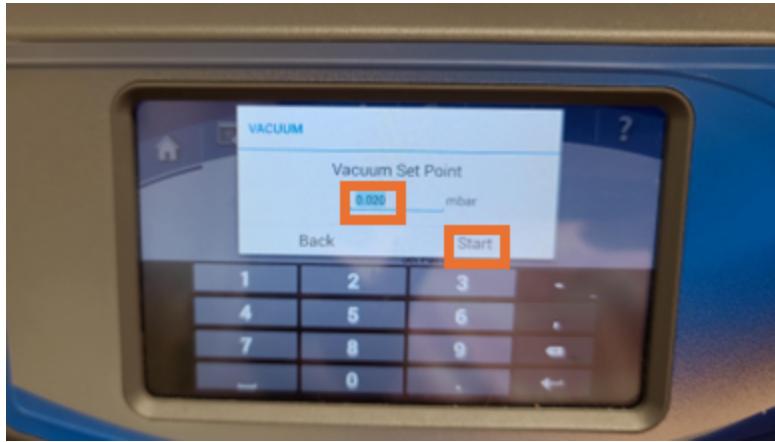
13.8 Use the touchscreen to select "Vacuum" from bottom left corner.



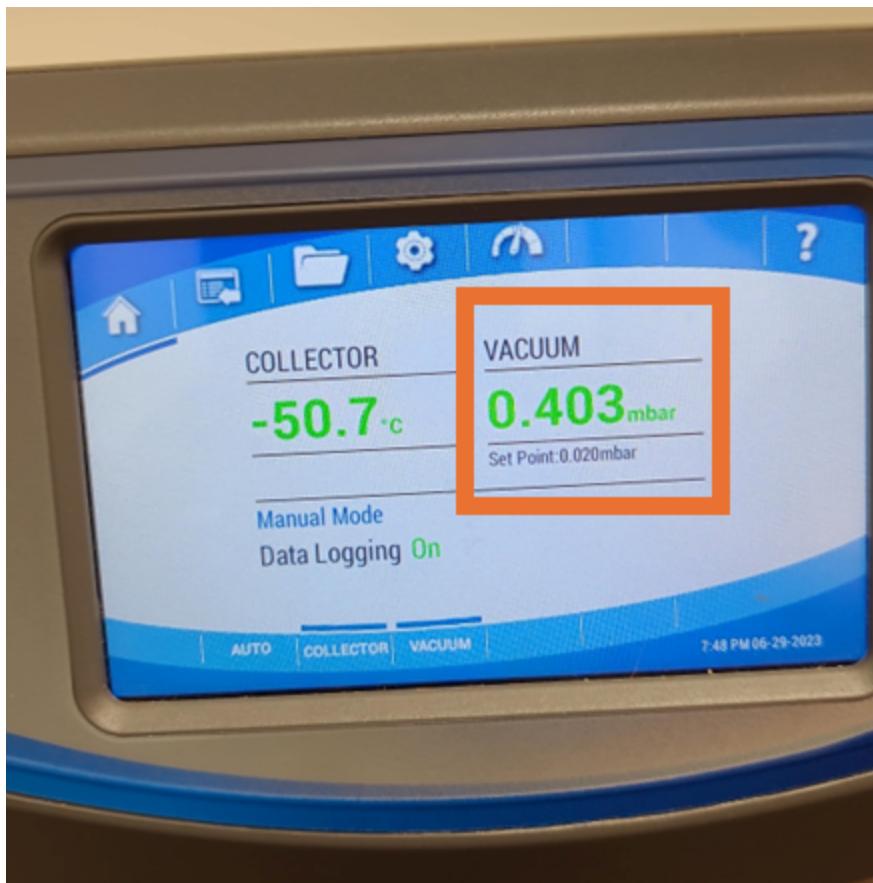
- 13.9 When prompted, use the touchscreen to set the pressure to 0.200 mbar and then press "Start" to start the vacuum. If a hissing noise is audible, recheck that all vacuum manifold ports are in the closed position. The pump motor should be audible at all times once the vacuum has been started.

Note

The vacuum will not be able to reach 0.200 mbar. By setting the vacuum set point so low, it ensures the samples are maintained under a constant vacuum for most effective drying and maintaining highest sample quality and integrity.



- 13.10 Wait for the vacuum pressure to turn from "High" to a red number and then from a red number to a green number (~<0.5 mbar). When both the collector and vacuum reading are displaying green numbers, the freeze-dryer is ready for use.



14 Transfer tubes from -80C to freeze dryer and lyophilize at <-40C and <0.5 mbar overnight. If a large number of samples will be prepared or if it is easier to keep samples in the racks during freeze-drying, a vacuum desiccator can be added to the freeze-dryer to increase capacity up to 3 full collection tube racks.

14.1 Remove the vacuum desiccator from storage in the cabinet below the freeze-dryer.

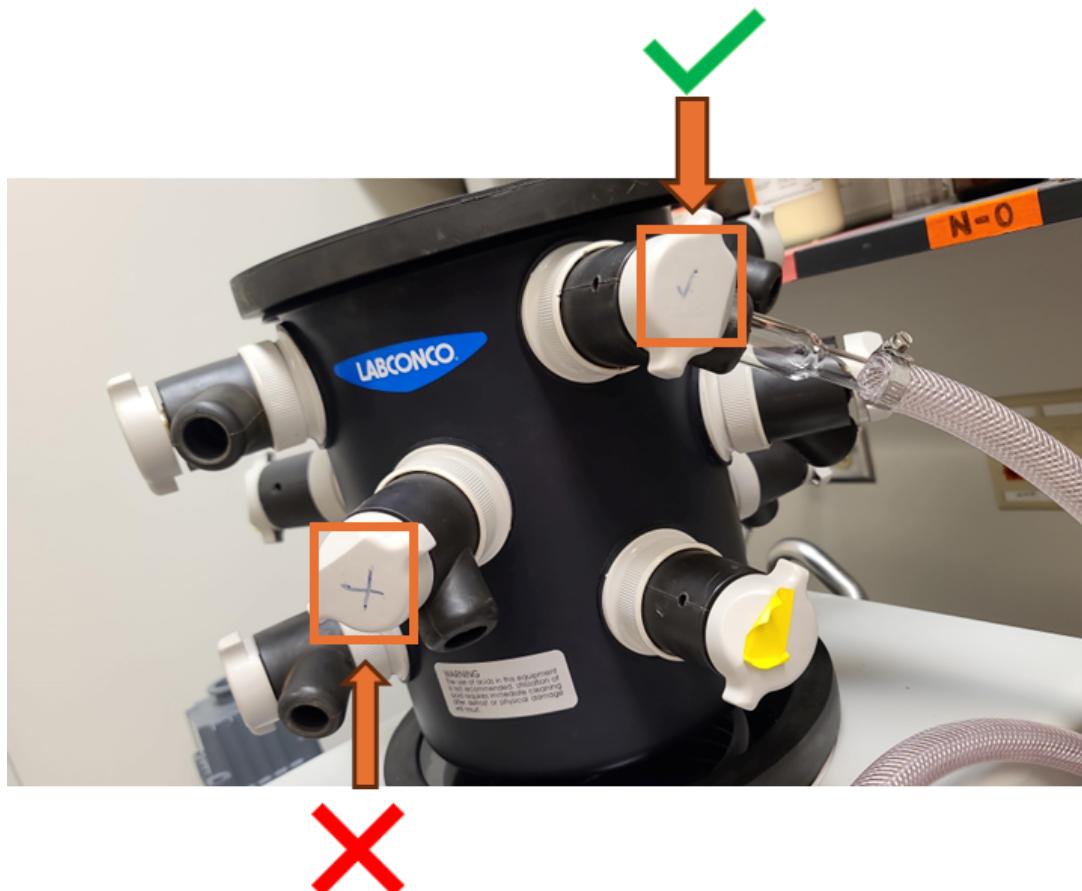


- 14.2 With the vacuum manifold port still in the off/vent position, push the glass adapter for the desiccator attachment hose into the rubber vacuum tube port.

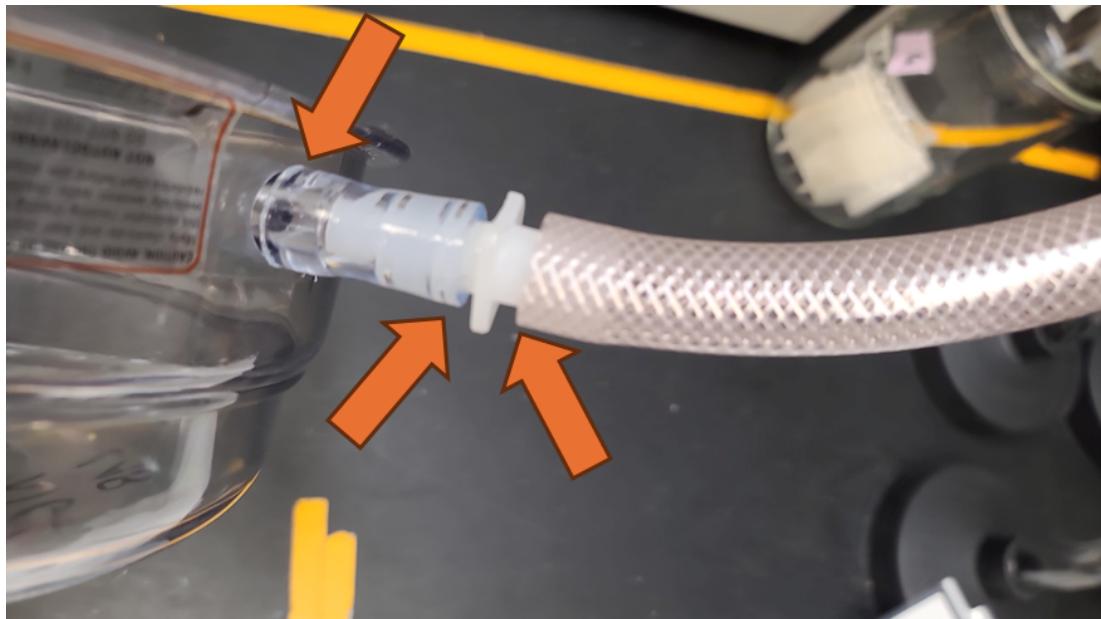


Note

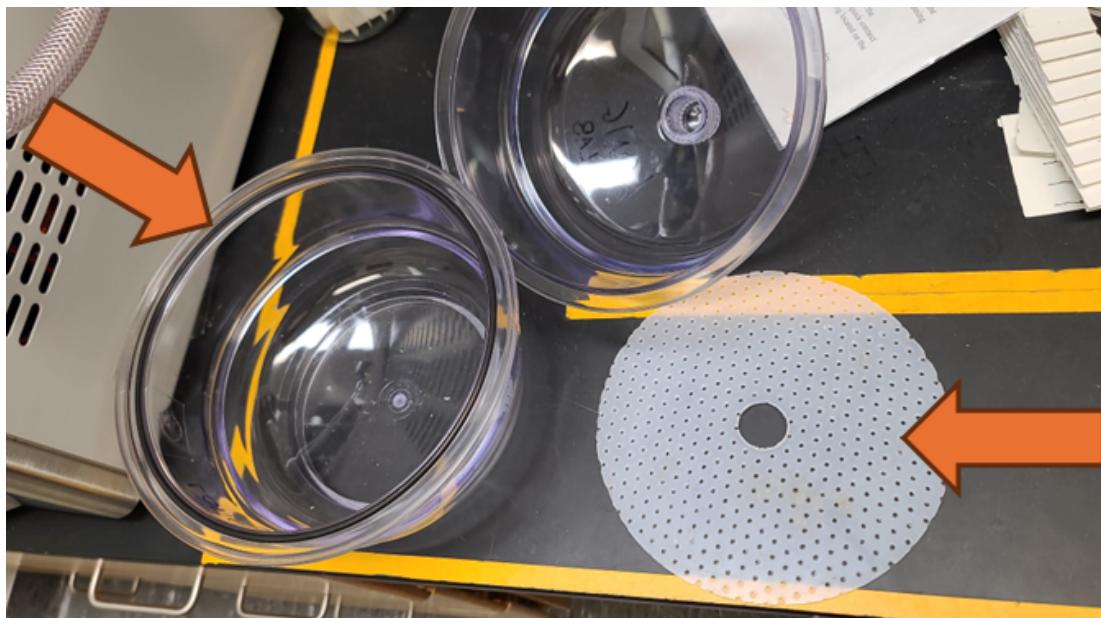
Not all vacuum manifold ports are functional. Only use ports with a check mark or no marking. Do not use ports with an X marked across them.



- 14.3 Ensure the other end of the tube is firmly pushed on to the barb fitting on both sides and on to the attachment port for the desiccator lid.

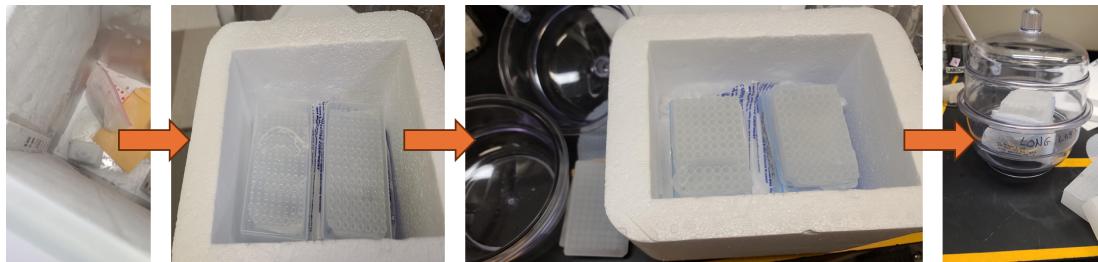


- 14.4 For additional room in the desiccator chamber, remove the white mesh shelf and set aside. Ensure the black gasket that goes between the desiccator and it's lid is present and in good condition.



- 14.5 Move the frozen samples from the -80C freezer to the desiccator on dry ice. Remove clear plastic collection box lid and set aside so only vented parafilm remains. Stack collection boxes

in desiccator up to three high. Replace desiccator lid.



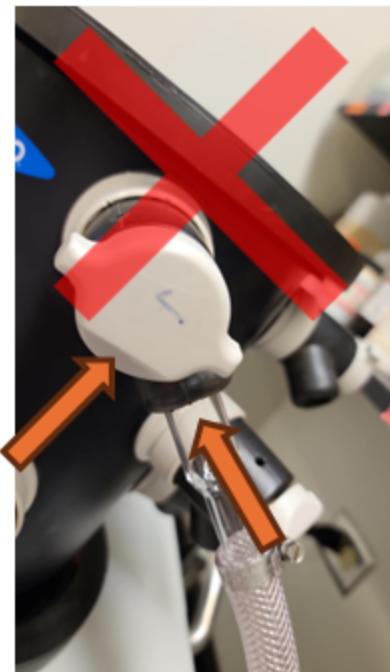
- 14.6 Open the appropriate vacuum manifold port on the manifold to apply vacuum to the desiccator. Vacuum ports are open when the middle of the flat portion of the port handle is pointing directly at the middle of the attachment tube. The vacuum reading on the home screen will go from low pressure/green to "High"/red as the vacuum initially has to remove the air from the desiccator but will return to low pressure/green within 5-10 minutes as vacuum is successfully applied.



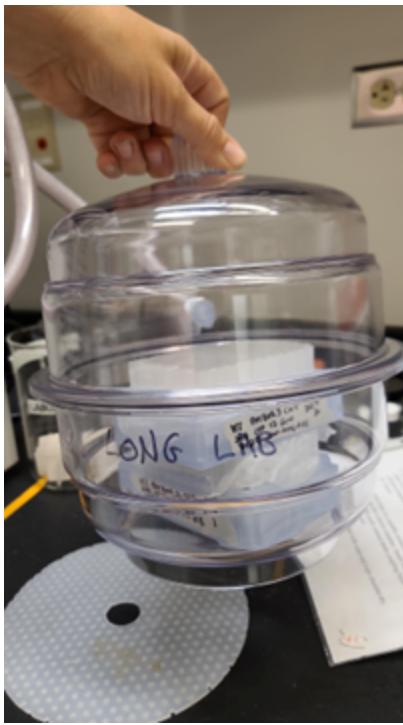
Note

Do not turn the vacuum manifold port handle past the vacuum hose attachment. The vacuum readings on the screen may look like they followed the appropriate reading on the home screen with a transition from green to red to green but the vacuum is only applied to the desiccator and samples are only freeze-dried when the middle of the flat portion of the handle aligns with the vacuum port.

***Condensation should not accumulate in the desiccator. If condensation is occurring, the samples are not under vacuum or not under sufficient vacuum and temperature to facilitate high quality freeze drying. ***

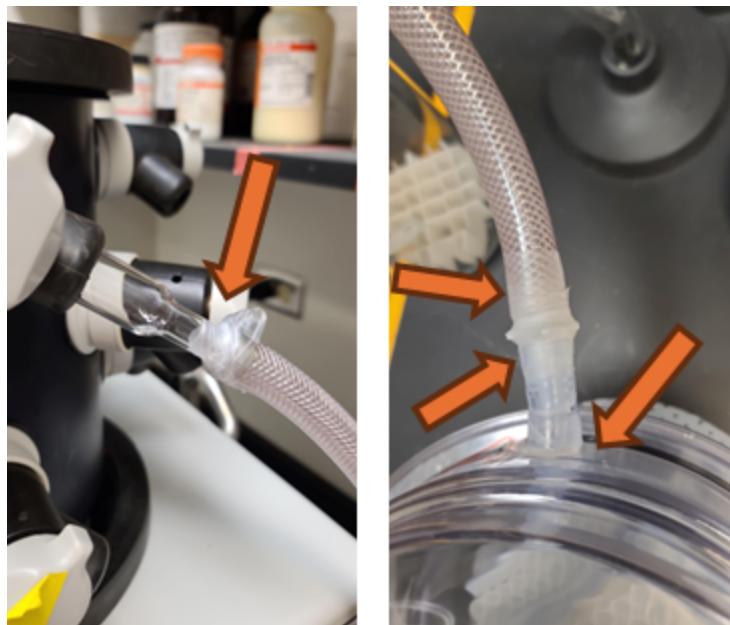


A good test to see if the vacuum has been properly applied to the desiccator is to pick up the desiccator by the lid. If it is not under vacuum, the lid will lift off. If it is under sufficient vacuum, it will be possible to lift the entire desiccator using the lid.



Note

If after 10 minutes, a vacuum reading in green is not reached, the vacuum manifold port handle is in the correct position, and/or an air leak is audible, seal all desiccator tube connections with parafilm.



- 15 Lyophilize at <-40C and <0.5 mbar overnight.

Note

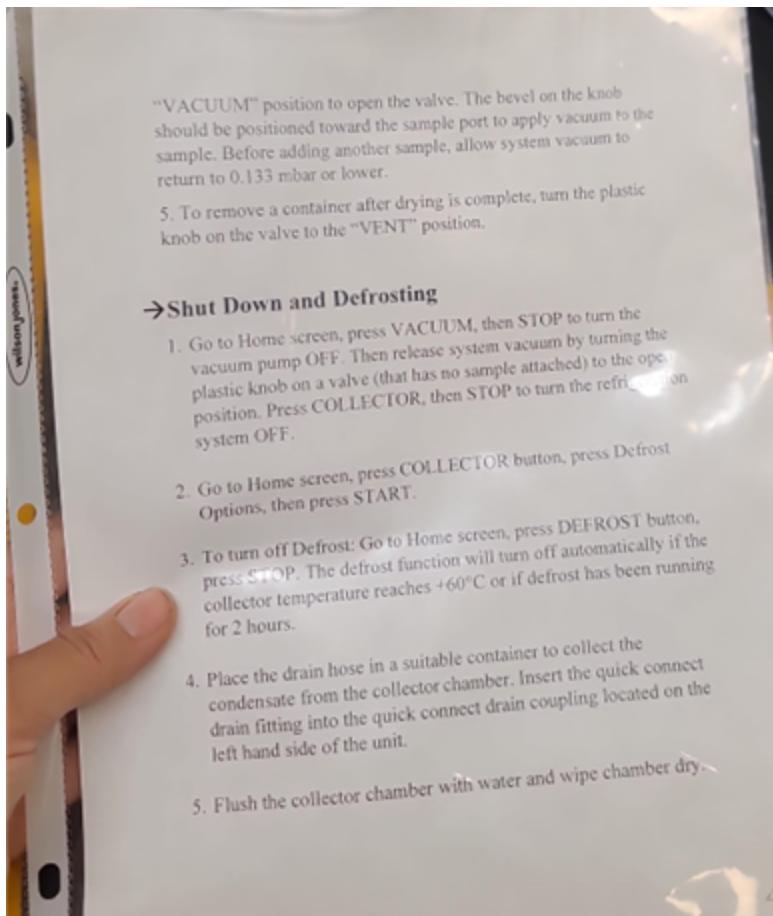
Freeze dry samples to steady state weight. For three racks of leaf tissue prepared as described above, overnight is sufficient. Modifications to tissue type or quantity sampled, may require adjustments to freeze drying time.

- 16 To release the vacuum from the desiccator, rotate the vacuum manifold port handle so that the middle of the flat portion of the port handle is pointing directly at the vent hole, directly opposite the middle of the attachment tube. A hissing of air being released should be audible.

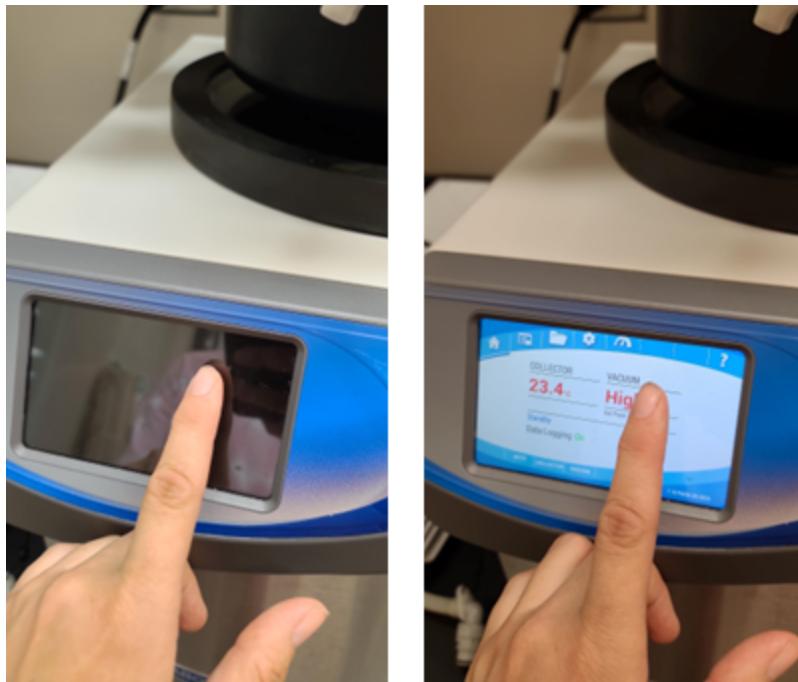
This will only release the vacuum on the desiccator but will maintain vacuum on the remainder of the freeze dryer manifold. The vacuum readings should remain green but the lid will lift off the desiccator after the vacuum has been vented, the hissing of air should cease.



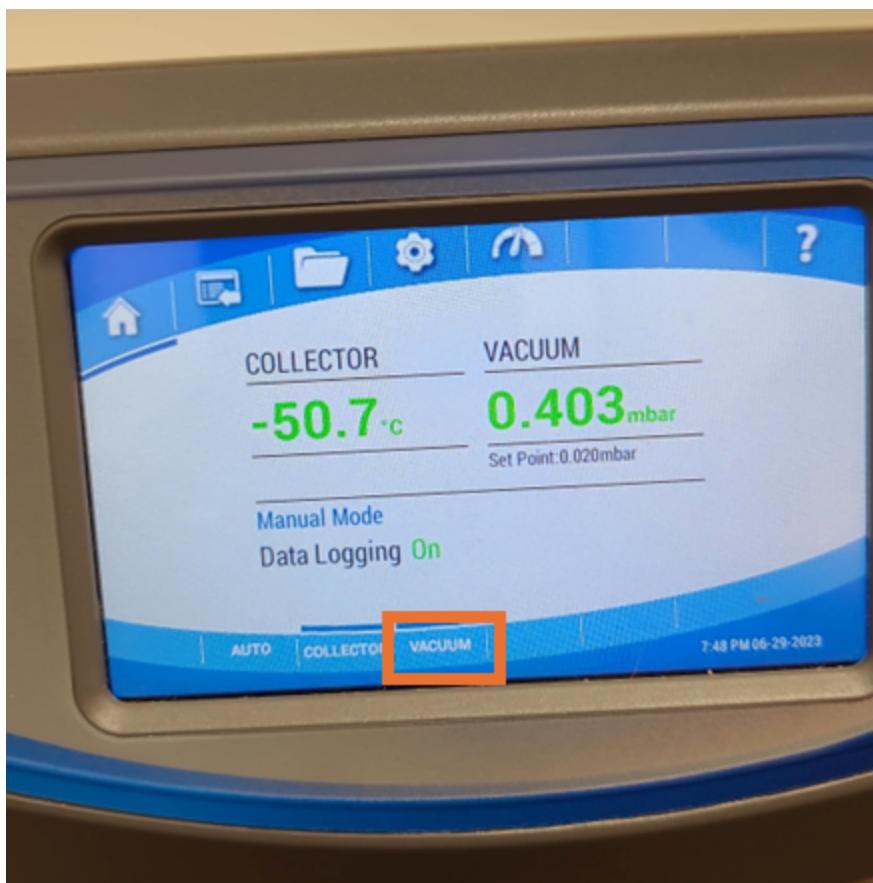
- 17 Shutdown freeze-dryer by warming, releasing vacuum, and removing residual water based on manufacturer's and lab manager's instructions.



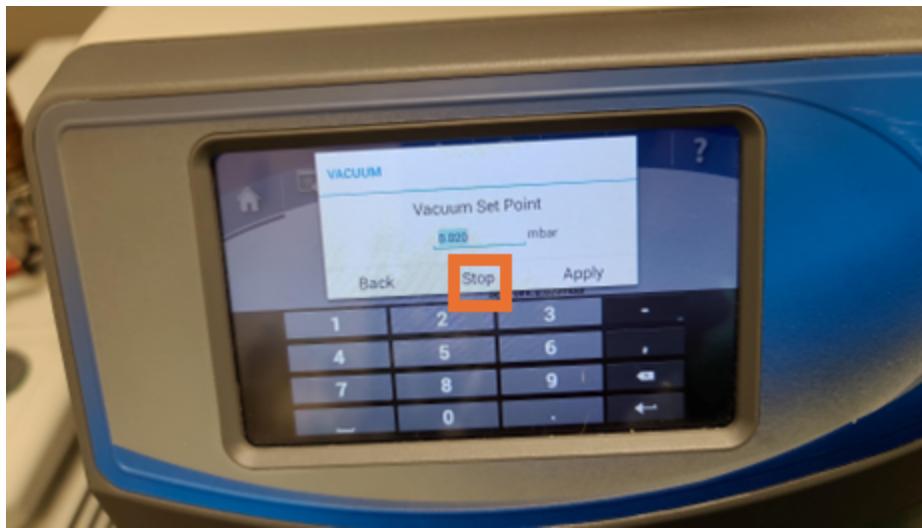
- 17.1 Press anywhere on the touch screen to wake up instrument.



17.2 Use the touchscreen to select "Vacuum" from bottom left corner.



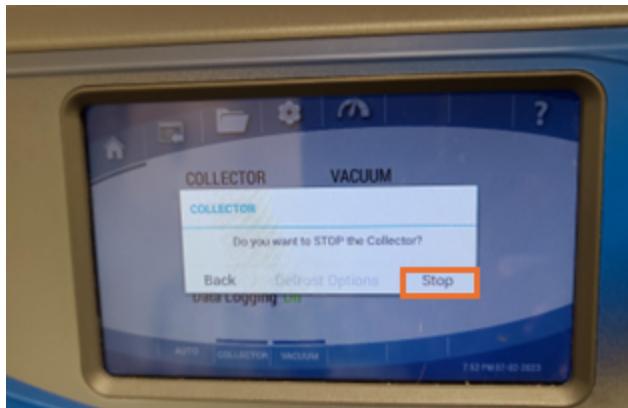
- 17.3 When prompted, use the touchscreen to press "Stop" to stop the vacuum. The pump motor should no longer be audible.



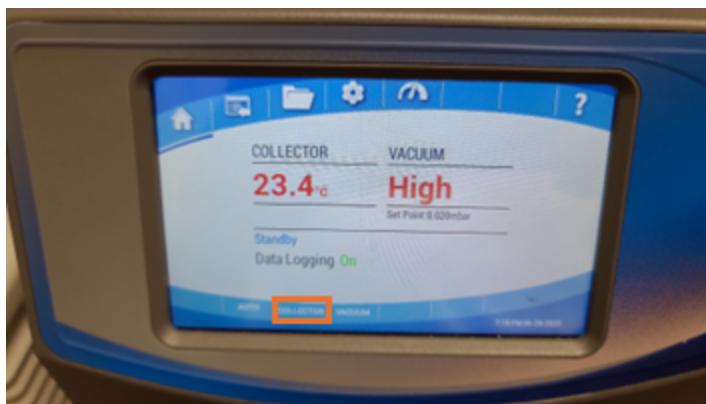
- 17.4 Use the touch screen to select "Collector" at the bottom left of the screen.



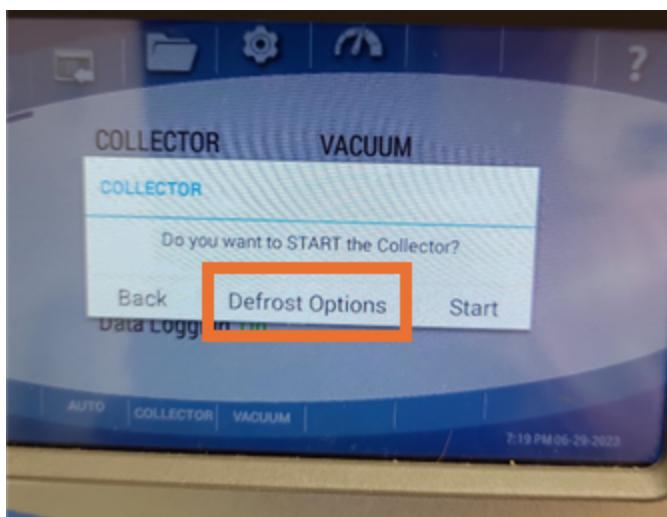
- 17.5 When prompted, use the touchscreen to press "Stop" to stop the collector.



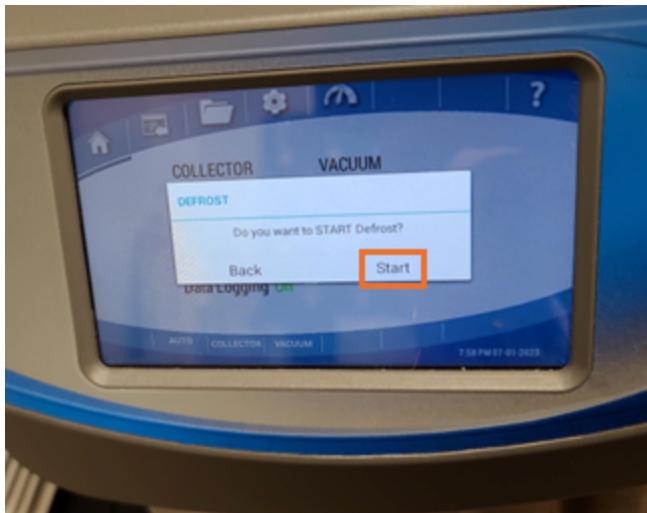
- 17.6 To start defrost, use the touch screen to select "Collector" at the bottom left of the screen.



- 17.7 When prompted, use the touchscreen to press "Defrost Options".



- 17.8 When prompted, use the touchscreen to press "Start" to begin defrosting the collector.



- 17.9 The defrost will turn off automatically if the chamber reaches 60C. The amount of water removed from copy number samples prepared as described above is minimal. The defrost usually turns off automatically in less than five minutes. If it does not, follow the lab manager's Quick Shutdown Guide.

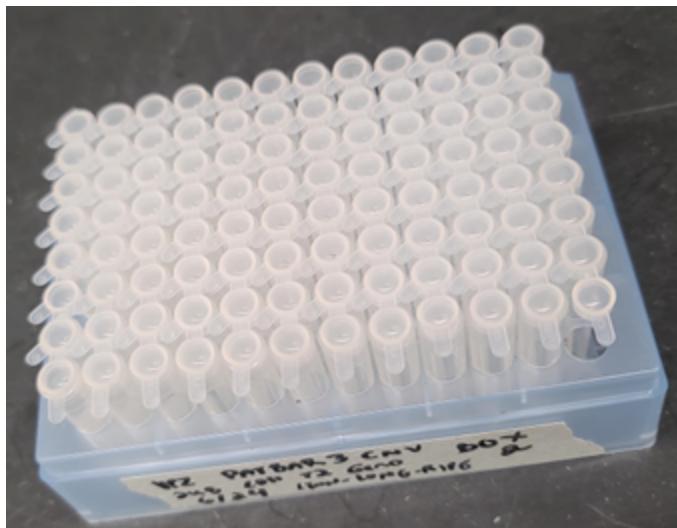
- 17.10 Remove the vacuum manifold and set it on the counter.



- 17.11 Dry the inside of the collector. The amount of water removed from copy number samples prepared as described above is minimal and the collector can easily be dried using paper towels. If a large amount of water is present, follow the lab manager's Quick Shutdown Guide.



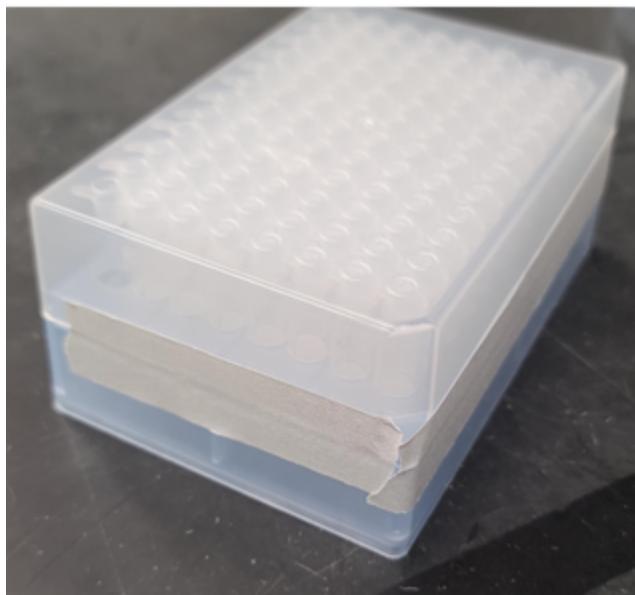
- 18 Remove samples from freeze dryer desiccator attachment, remove parafilm, and cap tubes using strip caps.



Note

Remove parafilm slowly as freeze-dried tissue can be staticky and may stick to parafilm or try to jump out of tubes.

- 19 Replace tube rack lid and tape closed.



Prepare Samples for Shipment

- 20 Clearly label the side of the box, on the box or tape, with the lab name, date, sample tissue type, and gene requested for copy number.
- 21 Prepare the [shipping documents](#).
- 22 Weigh the sample box and record the weigh on the commercial invoice form. The number of packages refers to the number of exterior boxes (typically 1). The quantity refers to the number of items in the package, typically we use the number of sample tubes for this value. For unit value, we typically assign each sample tube the value of \$1.
- 23 Give the package and shipping documents to [IGB Shipping Department](#) to review. If their advice differs from the above, heed their advice instead.
- 24 Forward shipping tracking information from IGB Shipping Department on to iDNA Genetics. If you had not previously sent them the completed sample template, "96 well template_iDNA.xlsx", send it with the tracking information.
- 25 Typically it takes 5-9 days to make it through customs. If tracking indicates a clearance delay for more than a few days, contact the UIUC/IGB purchaser and/or iDNA genetics to help move the package through export/import clearance.