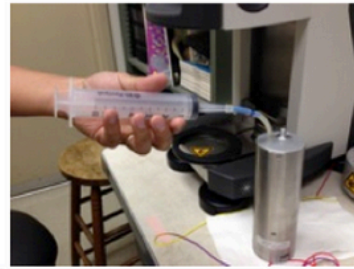


Vitrobot™ Standard Operating Procedure for cryo-sample preparation

1. Wear lab coat and appropriate gloves (cryo gloves when dispensing liquid nitrogen (LN2); latex or nitrile gloves when handling filter paper, grids, and samples to protect from contamination of oils on skin).
2. Connect the water cylinder to the humidity chamber.
Fill the water cylinder with DI water. Draw up to 55 ml of distilled water into the syringe and inject the water into the tube connected to the water cylinder (**Over filling the water cylinder can lead to over heating and burning of the plastic piece to which the cylinder mates on the Vitrobot**). Keep the syringe attached and pull 10 ml “air” out of the water cylinder. This results in a slight vacuum and now the humidifier is properly filled and will work properly.
3. Check to make sure all cables and wires are properly connected.
4. **Turn on and adjust the pressure of the Nitrogen gas tank to 70 psi.**
5. Turn on the switch on the back of the Vitrobot.
6. The Vitrobot User Interface has 2 pages: Console and Options.
 - (a). The console page is where the temperature and humidity are set.
 - (b). The options page is where the blot time, wait time, blot force, and blot total are set. This is also where the user specifies using the footpedal, turning the humidifier off during manual application and plunge freezing, and turning off the semi-automatic grid transfer.

*** Note: The 3 or 4s blot time usually yields good ice thickness but it depends on the sample; 3-4 ul is a good amount for a grid; drain time is usually set to 0-1s; Blot offset is usually set to -2 ~ -3 mm.
7. Remove the plastic rings from the Blotting Pads. Secure pieces of fresh filter paper to Blotting Pads using the clipping rings. Cycle through the reset blotting papers option to ensure good blot contact.
8. Begin to fill the Coolant Container with LN2.
9. Once the coolant container is cooled down (not boiling violently), position the metal spindle over the central cup. Open the main valve for the ethane cylinder and slowly condense the ethane gas in the central cup. The liquid ethane should reach the brim of the central cup for optimal vitrification.
10. Remove the metal spindle when you see ice begins to form. If you wait too long, the spindle will be hard to remove.

11. Attach the grid to the forceps/tweezers, move the black clamping ring down to the first groove on the forceps.
12. Mount the forceps to the Vitrobot by first selecting “Place New Grid” button (this moves the central axis into position for loading the forceps). Secure the forceps onto the connection groove in the central axis.
13. Select “Start Process” to move the forceps into the climate chamber (the foot pedal can also be used to advance to the next step).
14. Place the coolant container on the platform ring.
15. Select “Continue” (or step on the foot pedal) to raise the coolant container towards the climate chamber.
16. Select “Continue” (or step on the foot pedal) to lower the forceps slightly to allow for easier application of sample to grid through the side-entry port using a pipette (sample can be added from either side of the climate chamber, just remember to apply the sample onto the carbon side of the grid).
17. Select “Continue” (or step on the foot pedal) to activate blotting and plunge freezing, with any specified wait or drain times, and the lowering of the coolant container and the forceps.
18. Transfer the grid into pre-cooled grid storage box by carefully disconnecting forceps from the central axis. Moving the coolant container from the support ring to the bench top will facilitate transferring of the grid.
19. When all grids (maximum is 4) are placed in the storage box, tighten the screw to seal the grid box. Transfer the grid box to the large storage dewar.
20. Repeat steps 11-19 as needed to finish freezing all samples.
21. Turn off ethane and nitrogen tanks.
22. Place the coolant container in the fume hood for evaporation of LN₂ and liquid ethane.
23. To turn off the Vitrobot, press the “exit” button on the console screen. **Make sure there are no forceps attached to the central axis.** When the lights are turned off, central axis has moved into parking position, and the interface shuts down, then the toggle switch located at the back can be turned off. Close gas Nitrogen valve.
24. Disconnect the electrical cable from the humidifier by pressing up on the ring near the cable and pulling down the cable connector. Subsequently, twist and pull the humidifier to release the bayonet connection from the Vitrobot. Empty the reservoir by pouring the water from the central reservoir. Re-connect the syringe and withdraw the rest of the water from the outside reservoir. Leave the empty humidifier on the table and let it dry out completely. **Empty the Humidifier - All remaining water must be removed!**



Re-connect the syringe and withdraw the rest of the water from the outside reservoir.



Leave the empty humidifier on the table to dry out completely.

25. Fill out the logbook

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