

Problems? Do not attempt to fix them by yourself, find or call Misha or Mike. Vitrobot is very expensive, as well as downtime.

Vitrobot is an automatic cryo-plunger that is computer controlled. It has an environmental chamber allowing to control humidity and the temperature from which the grids are plunged to liquid ethane.

1. Check that the blotters are set apart and not touching each other.
2. Turn on the Vitrobot using switch on the back of it.
3. That turns on controlling computer and starts up the program. Main rod comes down through the blotters that must stay apart at that point.
4. Put DI water in the humidifier if you are going to use it (**that is rarely needed for Vitrobot 5**). Do not overfill.
5. Put aluminum spider onto brass cap for ethane. Put grid box into a slot of the holder, make sure that the lid rotates freely, if not, loosen the screw. Add liquid nitrogen into plastic box, keep it high enough for the lower part of the spider to be immersed in nitrogen. That cools down the ethane cap. Wait until the cap is cold enough (3-5 min), then open ethane cylinder and the closest to Vitrobot ethane valve (slowly), start filling ethane into the cap. Do not overfill, ethane expands upon solidification and will mess up everything around, when it gets to liquid nitrogen, you will hear “pops”. It does obscure liquid nitrogen surface as well; try to prevent that from happening.
6. Protective plastic shield helps to keep everything frost-free in the freezing box. It floats in the nitrogen providing dry cold blanket of nitrogen gas above the grid box.
7. Put the box in the holder at the bottom of the Vitrobot.
8. Check the program, load user settings, set temperature, humidity and blotting duration in the corresponding widgets. Usually one time blotting for 1-3 s is enough for our specimens. We do not use bulk specimen application by dipping grids into an Eppendorf tube, uncheck that box. You need to apply the sample using pipettman through the side hole. Check that the front door of environmental chamber is closed.
9. Initiate program sequence, go to “mount tweezers”
10. Pick up a grid with tweezers, clamp it and mount tweezers onto the rod
11. Next step draws tweezers up to the chamber
12. You could apply the sample now to the grid
13. Next step will cause blotters to squeeze the grid for the preset amount of time and drop grid into ethane. Carefully remove tweezers from the rod and transfer the frozen grid to the cryobox.
14. Repeat with next grid.
15. After the session is finished exit the program following prompts. Close the chamber’s door. Shut down Vitrobot. Close ethane cylinder valve, otherwise you will find the cylinder empty next time. Discard liquid nitrogen from freezing box, remove ethane cap from the box to warm it up.