

Vitrobot SOP

1. Turn ON dry nitrogen supply (this is necessary to ensure that the Vitrobot works).
2. Turn ON the Vitrobot by using the switch on the bottom right corner at the back of the Vitrobot.
3. Screen will come up, acknowledge any messages about day-light savings time.
4. The VR application should start, but if not, double-click on the VR icon on the desktop.
5. Fill water reservoir with Mili-Q water and mount the reservoir. The position of the reservoir is important for the proper lifting of the nitrogen platform.
6. Put in place new filter papers and make sure the door is completely closed.
7. Dial in all your parameters on the main screen and on the Options screen (blotting time, tweezer offset, drain time, etc).
8. Go through the plunging sequence once to verify that water reservoir is in place and that all is working well.
9. Reset blotting paper position to 0.
10. Cool down the liquid nitrogen and liquid ethane reservoir. Be mindful of dispensing the cryogenes.
11. Mount the grid on the tweezers and click Continue, this will bring the Vitrobot arm into position to mount the tweezers.
12. Mount the tweezers and click Continue. This will lift the tweezers and bring them into the chamber. Click Continue to bring the liquid nitrogen/liquid ethane reservoir to the chamber.
13. Click Continue, this will bring the tweezers down enough to as to apply the protein/sample solution to the grid.
14. Click continue and monitor the plunging event, making sure that all goes smoothly.
15. The grid will be dropped into liquid ethane after the specified blotting time.
16. Carefully unmount the tweezers from the arm and quickly (but securely) transfer your grid to the storage box.
17. Repeat steps 11-16 for all your grids.
18. When you are finished:
 1. Clean up the area
 2. Dump the water on the reservoir and set it to the side to dry
 3. Take liquid ethane to the hood. Dispose of the liquid nitrogen and set the reservoir on dry paper towels and away from electronics.
 4. Remove filter papers and dispose of them properly.
 5. Label your tube and store your sample in the appropriate sample dewar.
 6. Click exit on VR user interface. The application will ask you if you have or have not removed the tweezers. Choose appropriate option (ie. the

tweezers removed and safely stored).

7. Wait for the monitor to turn off and then turn off switch on Vitrobot.
8. Close the dry nitrogen regulator.
9. Clean up the area.
10. If no one else is in the lab, turn off the lights.