Vitrobot Instructions

1. **First, turn on lab ventilation!!**
2. Fill the humidifier with water. Use the big syringe and inject a max. of 60ml water into the tube, then remove the surplus water with the same syringe until only air comes out.
3. Turn on the Vitrobot and check if the compressor is pressurized (about 6 bars).
4. Go to “Options” and load file (“10s”)
5. Switch on the humidity to 100% and the temperature to 4°C. It might take a while until the desired values are reached. Make sure the humidity switch is set to ON! Re-check while working!
6. Change old blotting paper by removing the white plastic rings with fingers. Always use a double layer for each side.
7. Put the silver temperature conductor (“Spider”) into the black trough and fill N2 into the black N2 trough to pre-cool it (also fill N2 about 3 times into brass pot).
8. While cooling black trough with N2, glow-discharge grids for 30” at 2,2 $^{-1}$ torr.
9. Go to the fume hood to fill ethane into the empty (!) brass pot. Open ethane bottle (left) and valve on the right to check pressure. Don’t touch the valve in the middle. Close the right valve, hold the tip steadily into the bottom “corner” of the pot and carefully open valve again. Make sure, no ethane mixes into the N2 and the other way around.
   DO NOT CHANGE ANY SETTINGS OF THE ETHANE FLASK!!!
10. DON`T FORGET TO WEAR THE FACE SHIELD AND GLOVES FOR YOUR OWN AND YOUR SAMPLES PROTECTION!!!!
11. Place the trough underneath the Vitrobot and wait until ethane starts to turn white. Then take out the “Spider”.
12. Put pre-chilled grid box into the grid box place inside the trough and open it with the white grid box tool.
13. On the computer click “place new grid”.
14. Pick up a grid with the Vitrobot-tweezers and clamp them tightly. Slide the tweezers centrically (!!) onto square bit of the hydraulic metal rod. Make sure, that the orientation the grid is correct. (depending on if you are left-or right-handed)
15. Click “continue”, the tweezers will move up.
16. Choose “Start process” and pipette 3,5µl of sample through the small sliding door onto the grid. Don’t bend the grid by touching it directly.
17. Click “continue” and automatically incubate for 45” using the programmed wait time
18. After the 45”, the Vitrobot will blot for 10” and then quickly plunge the grid into the ethane. Turn humidity back on!
19. Refill N2.
20. Remove the tweezers from the metal rod by pressing your thumb against the rod and, with your other hand, pull the tweezers toward you. Be careful to keep the grid inside the ethane dish.
21. Move the tweezers into the cold phase of the N2 and quickly blot the surplus ethane off with blotting paper.
22. Slide the clamp that close the tweezers right to the top, but keep them shut tightly with your fingers to make sure that the grid doesn’t fall off.
23. Move the grid into the grid box and be sure it is inserted completely. Always stay within the cold N2 gas phase.
24. Close the grid box and put it into the storage falcon. Submerge it into N2 and store it inside the Taylor Dewar.
25. To turn off the Vitrobot, click “exit” and follow instructions of program.
26. **Shut down computer AND shut down Vitrobot** and cover it with the plastic bag.
27. Place still full ethane trough under fume hood to let it evaporate.