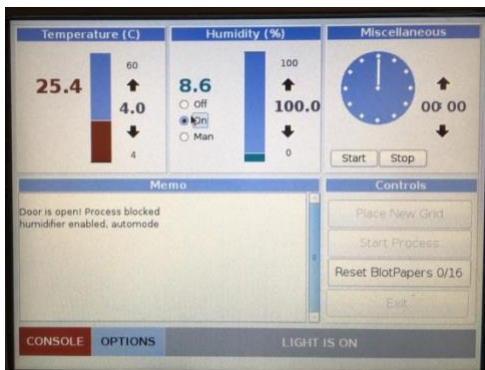
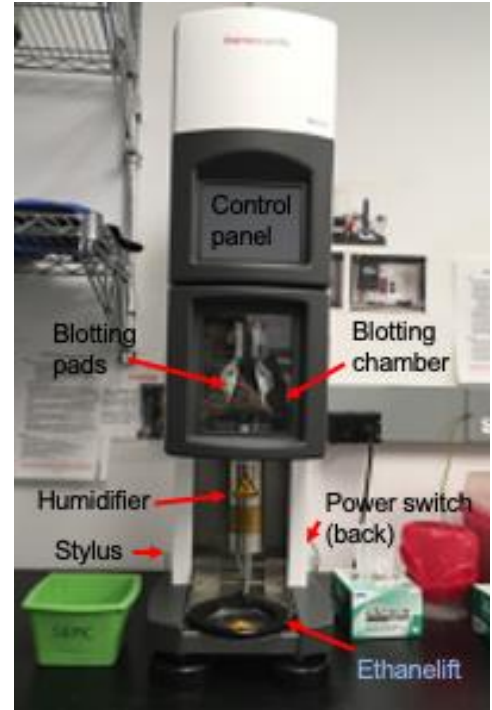


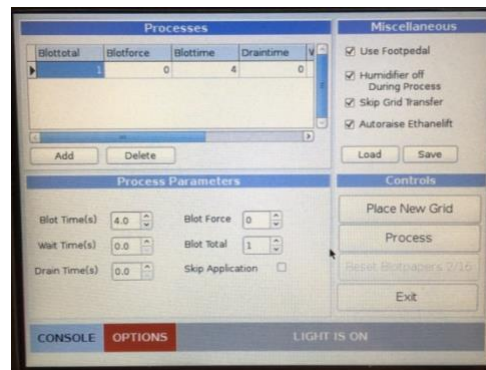
## Vitrobot plunge freezer manual

### Set-up

1. Mount the humidifier with the black dot facing the front.
  - a. Fill it with 60 ml of distilled water using a syringe via the rubber inlet.
  - b. After injecting all the water, draw out air from the humidifier into the syringe, then detach the syringe.
2. Switch on the power – the switch is behind the Vitrobot, on the bottom right side.
3. On the control panel, in the 'Console' page, set humidity and temperature to desired values.
  - a. Typically, 100% humidity and 4°C.
  - b. Select 'On' for Humidity.
4. In the 'Options' page, set your blotting process parameters.
  - a. 'Blot total' needs to be '1' for blotting to occur.
  - b. In the 'Miscellaneous' side bar, check 'Humidifier off during process' and 'Skip grid transfer'. Optionally, check 'Use footpedal' and 'Autoraise ethanelift'.
  - c. Set your desired blot time, wait time, drain (post-blot) time, and blot force. If unsure, start with 0 blot force, 0 wait time, and 2-4 s blot time.
  - d. Make sure 'Skip application' is unchecked.
5. Click on 'Place New Grid' and do a dry run through the process once.
6. Ensure grids are ready (i.e. glow discharged, if desired).

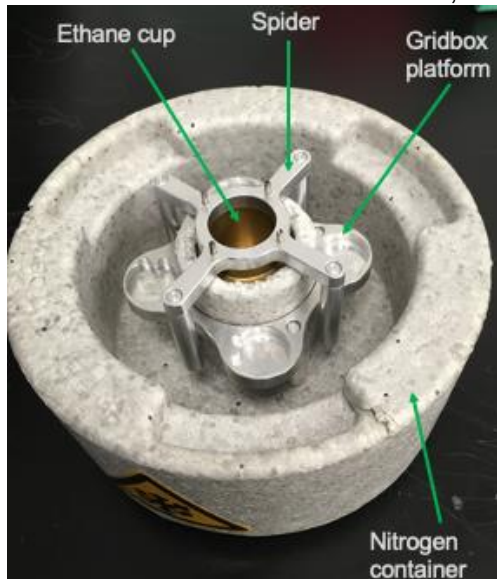


Console page, for controlling temperature and humidity



Options page, for controlling blotting parameters

7. Assemble the ethane container, then cool it down with liquid nitrogen.



8. Check that the ethane cup does not contain any liquid nitrogen before condensing ethane. Carefully condense ethane.
9. Place blot papers onto the blotting pads. Support the blotting arms when assembling the blot papers to prevent damage to the arms.
10. Once ethane is condensed, remove the spider, and place the foam ring and grid box into the container.
11. Attach Vitrobot tweezers with a grid onto the plunge rod. Use the Control Panel to bring up the tweezers into the blotting chamber.
12. Place ethane container onto the ethane lift, then lift it.
13. Click on 'Process', then apply sample to grid. Click on 'Continue' to blot and plunge.
14. Carefully detach tweezers from the plunge rod, then transfer grid to the grid box.
15. Dry / warm up tweezers, then repeat.