

# Vitrobot Plunge Freezer Protocol

## Setup:

- Attach the humidifier and fill with distilled water:
  - Fill syringe with 60 mL of distilled water (make sure there are no air bubbles) and fill the humidifier through the plastic tube at the bottom.
  - After injection, “de-fill” the humidifier by filling the syringe with air while still attached to the tube. This may have to be done a few times (detaching, emptying out the syringe, reattaching), until little-to-no water is observed in the syringe.
- Turn on power switch on bottom right.
- Replace blotting papers.
- Set humidity to 100 by pressing the up arrow (default is 70) and turn on.
- Press “options” using the stylus, and enable “use foot pedal” and “skip grid transfer.” The blot time can be adjusted as well as the wait time (time to wait after sample is placed on the grid before blotting).
- At this point, if you are not familiar with the steps of the foot pedal, you can test it out.
- Fill the cryo workstation with liquid nitrogen and use the “spider” to cool the ethane cup. Be sure to place the guard, which is kept in the drawer.
- Slowly condense ethane into cup. Once ethane begins to get frosty, remove spider.
- Attach Vitrobot tweezers with glow discharged grid onto the plunge rod.
- Place cryo workstation on Vitrobot and lift.
- Add sample to carbon side of grid at loading position and close side opening.
- Plunge freeze.
- Remove tweezers and quickly transfer grid from ethane cup to liquid nitrogen, then into a cryo grid box. When using the guard, it might be helpful to carefully move the workstation off the Vitrobot in order to see better.

## Shutdown:

- Close the ethane tank.
- Remove tweezers and press “Exit” then “yes.”
- Once computer screen is off, turn off the power switch.
- Detach humidifier, empty contents, and leave upside down to dry.
- Remove blotting paper and leave door open.
- Place the workstation in the storage bin in the fume hood.
- Empty LD4 and invert to dry.
- Return all tools to drawer and clean up.

**If you suspect any damage to the machinery, or encounter any problems during use, please tell Ashleigh or another EMG staff member.**