**Purpose**: To setup a Vitrobot Mark IV Plunge Freezer.
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## 1. Purpose:

1.1. To setup a Vitrobot Mark IV in an effective and safe manner.

## 2. Scope:

2.1. To safely setup a Vitrobot Mark IV and to shut it down.

## 3. Definitions

3.1. Shutdown of a Vitrobot Mark IV for the safety of the scientific device and considerations for the next user.
3.2. Setup of a Vitrobot Mark IV for the safe operation of the scientific device.

## 4. Responsibilities:

4.1. Attach the humidifier and fill with distilled water.
4.2. Fill the 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 or from the top.
4.3. After injection, “de-fill” the humidifier by filling the syringe with air while still attached to the tube.
4.4. 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.
4.5. Turn on the power switch on the bottom right.
4.6. Replace blotting papers.
4.7. Set humidity to 100 by pressing the up arrow (default is 70) and turn on.
4.8. Press “options” using the stylus and enable “use foot pedal” and “skip grid transfer.”
4.9. The blot time can be adjusted as well as the wait time (time to wait after a sample is placed on the grid before blotting).
4.10. At this point, if you are not familiar with the steps of the foot pedal, you can test it out.
4.11. Fill the cryo workstation with liquid nitrogen and use the “spider” to cool the ethane cup. Be sure to place the guard, please refer to Figure 1.
4.12. Slowly condense ethane into the cup, you will hear a gurgling sound when the gas turns into liquid. Once ethane begins to get frosty, remove the spider.
4.13. Attach Vitrobot tweezers with glow discharged grids onto the plunge rod. Place cryo workstation on Vitrobot and lift.
4.14. Press foot pedal once for plunge rod to rise up with tweezer and sample.
4.15. Add a sample to the grid either through the right slot or left slot.
4.16. Press foot pedal to plunge the grid.
4.17. Remove tweezers from the plunge rod and quickly transfer the grid from ethane cup to liquid nitrogen, then into a cryo grid box.
4.18. When using the guard, it might be helpful to carefully move the workstation off the Vitrobot in order to see better.
4.19. The guard from the cryo workstation can be left on or depressed downward for better visibility when transferring the grid to the grid box.
4.20. The shutdown of the Mark IV will start with closing the ethane tank.
4.20.1. Remove tweezers and press “Exit” then “yes.”

4.20.2. Once the computer screen is off, turn off the power switch.
4.20.3. Detach humidifier, empty contents, and leave upside down to dry in and ice bucket.
4.20.4. Remove blotting paper and leave the door open.
4.20.5. Place the cryo workstation in the storage bin in the fume hood. This done so, the ethane in the cup will evaporate in a safe manner.
4.20.6. Empty LD4 and invert to dry.
4.20.7. Return all tools to the drawer and clean up..

**5. Personal protective Equipment (PPE):**

5.1. Laboratory coat
5.2. Nitrile gloves
5.3. Goggles
5.4. Cryogenic gloves
5.5. Face mask

**6. Chemicals:**

6.1. Ethanol 70%

**7. Equipment**

7.1. Liquid Nitrogen (LD4 or LD5)
7.2. Tweezers
7.3. Small Screwdriver or Gripper Tool for your cryo grid box
7.4. Cryo Grid Box
7.5. Transfer Dewar
7.6. Pipette
7.7. Pipette tips
7.8. ddH2O
7.9. Sample
7.10. Plasma Cleaned Grids
7.11. Filter Paper

**8. Waste Disposal:**

8.1. N/A

**9. Vendors:**

9.1. N/A

Figure1: