

Vitrobot Checklist

Supervising Staff: _____
Trainee(s): _____

Date: _____

Preparing the Vitrobot

- Attach humidifier to Vitrobot, sticker facing forwards.
- Fill humidifier with ___ mL water. Draw back on the syringe to create vacuum in humidifier chamber.
- Place new filter paper on the blotting pads.
- Turn on the Vitrobot
- Set humidity to __%. Set temperature to ___ C.
- Set process parameters.

Cooling the Ethane

- Assemble ethane holder.
- Fill with LN2.
- When all metal has cooled to LN2 temperature, prepare to add ethane.
- Add ethane to central brass cup. Fill to bottom of spider.
- Let ethane cool until a thin layer of ethane frost coats the inside of the ethane cup.
- Remove the spider.

Freezing Cycle

- Set humidity to __%.
- Place new grid-box under LN2, in holder.
- Pick up glow-discharged grid with the Vitrobot tweezers.
- Select start process (or use foot pedal) to lower the plunge-rod.
- Attach tweezers to the plunge-rod.
- Press next step (or foot pedal) to raise plunge-rod into freezing chamber.
- Place ethane holder onto the ethane lift.
- Press next step (or foot pedal) to raise ethane holder.
- Press next step (or foot pedal) to lower the grid to sample loading position.
- Add sample to grid by placing pipette through loading port on the side of freezing chamber that carbon is facing.
- Press next step (or foot pedal) to start the plunging process. Wait for process to end and everything to be lowered fully.
- Unclip Vitrobot tweezers from plunge-rod and move ethane holder off of the lift onto the lab bench.
- Place frozen grid in available grid-box slot.

Revitalizing the Ethane

- Place a room temperature spider upside down on the ethane cup.
- Wait for ethane to thaw.
- Place cold spider on ethane cup with legs in LN2. Wait for ethane frost to coat inside of the ethane cup.

Cleaning Up

- Ensure the Vitrobot forceps are dry and put away.
- Tap the Exit button the the Vitrobot screen.
- Wait for the Vitrobot to shut down: the screen will go completely blank.
- Flip the power switch at the back of the Vitrobot.
- Remove the used filter papers.
- Remove humidifier and dump out water by inverting the chamber several times.
- Place the humidifier upside-down in a clean and dry container for storage.
- Make sure the ethane tank valves are closed.