



Cryo-EM Merit Badge Independence Checklist

A Collaboration Among the NIH-Funded Cryo-EM National Centers

Vitrobot Independence Test Checklist – CryoEM Merit Badges

Trainee: _____

Preparing the Vitrobot

- ☐ ☐ Attach humidifier to Vitrobot, sticker facing forwards.
- ☐ ☐ Fill humidifier with ____ mL water. Draw back on the syringe to create vacuum in the 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 the bottom of the spider.
- ☐ ☐ Let ethane cool until a thin layer of ethane frost coats the inside of the ethane cup.
- ☐ ☐ Remove the spider.

Freezing Cycle

- ☐ ☐ Check that temperature and humidity have reached target.
- ☐ ☐ 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 the process to end and everything to lower 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 the 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.

Supervising Staff 1: _____

Date: _____

Supervising Staff 2: _____

Date: _____