



Vitrobot Independence Test Checklist Study Guide – CryoEM Merit Badges

Supervising Staff: _____

Date: _____

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.
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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.
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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.
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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.
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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.
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