



Vitrobot Independence Test Evaluator Checklist – CryoEM Merit Badges

Supervising Staff: _____

Date: _____

Trainee: _____

Preparing the Vitrobot

- Identify the following:
 - Power Switch
 - Screen
 - Humidifier
 - Environmental/Freezing Chamber
 - Ethane Lift
- Attach humidifier to Vitrobot, sticker facing forwards.
- Fill humidifier with ___ mL water.
- Draw back on the syringe and clear excess water from line.
- Place new filter paper on the blotting pads.
- Turn on the Vitrobot
- Set humidity, temperature, and process parameters:
 - Humidity: ___% RH
 - Temperature: ___ °C
 - Blotting Time: ___ s
 - Wait Time: ___ s
 - Blot Force: ___
 - Skip Grid Transfer: yes/no
 - Use Foot Pedal: yes/no
 - Auto-raise Ethane Lift: yes/no
 - Skip Botting Position: yes/no

Evaluator Notes:

Cooling the Ethane

- Identify the components of the ethane holder:
 - Spider
 - Ethane Cup
 - Grid-box Holder
 - Base
 - Anti-Contamination Ring
- Assemble the ethane holder.
- Fill base with LN2.
- Fill ethane cup with ethane.
- Let ethane cool until a thin layer of ethane frost coats the inside of the ethane cup.
- Remove the spider.

Evaluator Notes:



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 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.

Evaluator Notes:

Revitalizing the Ethane

- Place a spider upside down on the ethane holder.
- Wait for ethane to thaw.
- Place cold spider on ethane cup, and wait for ethane frost to coat inside of the ethane cup.

Evaluator Notes:

Cleaning Up

- Ensure the Vitrobot forceps are dry and stored with the tip protector attached.
- Shut down the Vitrobot.
 - Wait for the Vitrobot to shut down: the screen will go completely blank.
 - Flip the power switch at the back of the Vitrobot.
- Remove and dispose of 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.

Evaluator Notes:

Overall Evaluator Notes:
