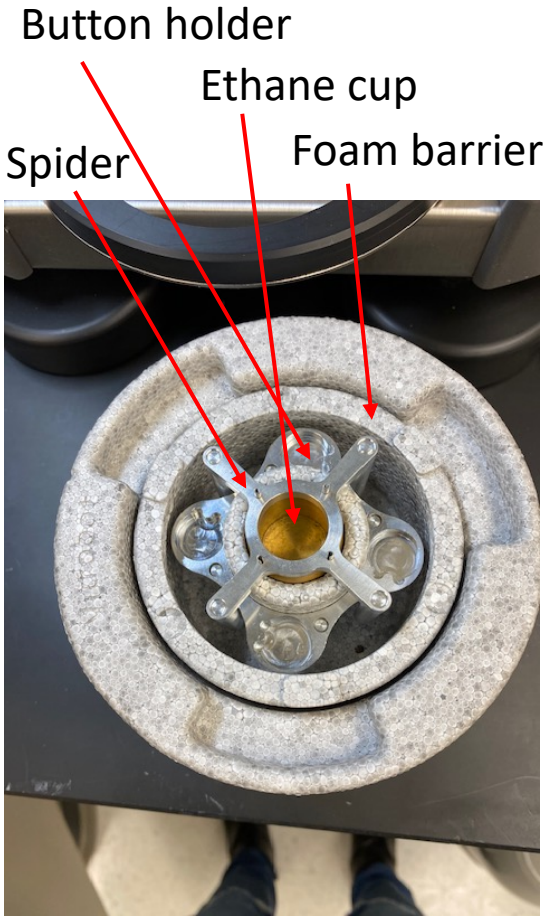
Vitrobot
MARKIV
Fred Hutch

Inventory & assemble freezing container

Sample and grids(!), small/medium/large tweezers, screwdriver, pipet and tips, glow discharge slide, safety glasses, freezing container and LN2 containers (fill 4L container from LN2 tank)



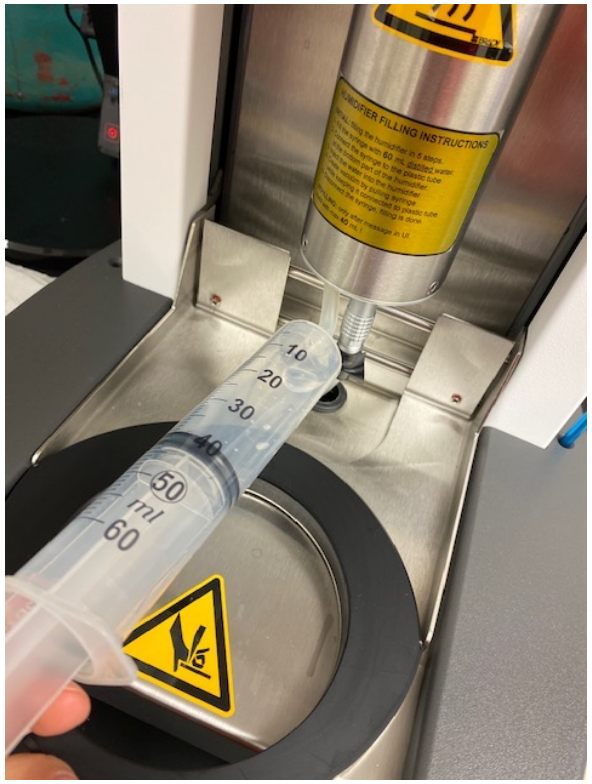
Attach and fill humidifier

Flip humidifier upside down, line up red dots and push to attach

Careful with cord!

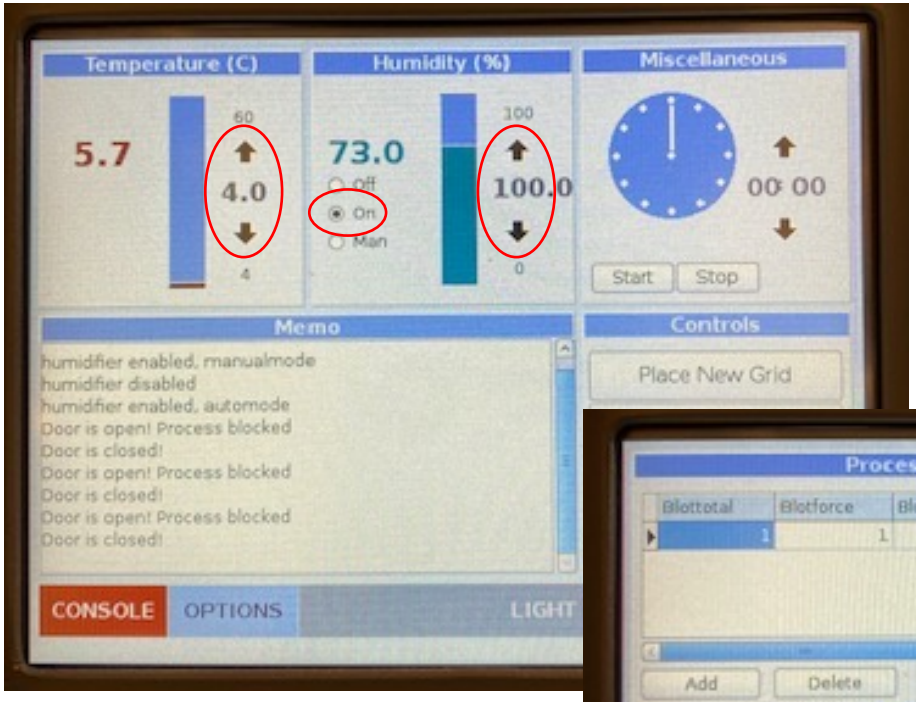
Line up pins and grooves, push up and turn counterclockwise

Yellow sticker to front!



Inject 40 mL of dH2O into humidifier and then pull back syringe to empty line before detaching

Setup console, options, & label button

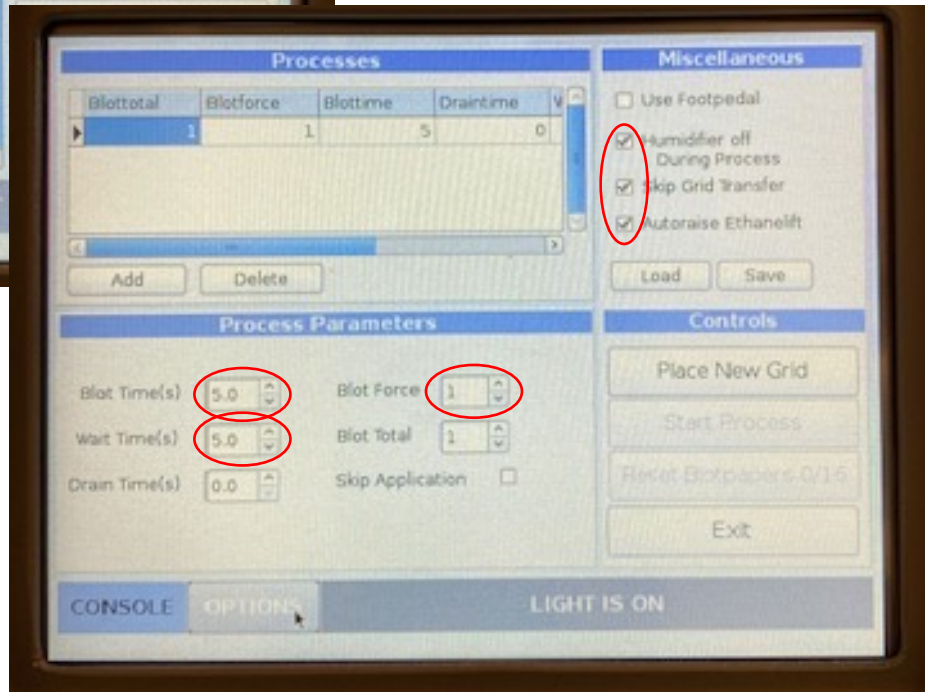


Console:

- Adjust temp with arrows
- Adjust humidity with arrows
- Humidifier on

(Humidifier will not adjust until it reaches your set temp)

Label button on side and bottom



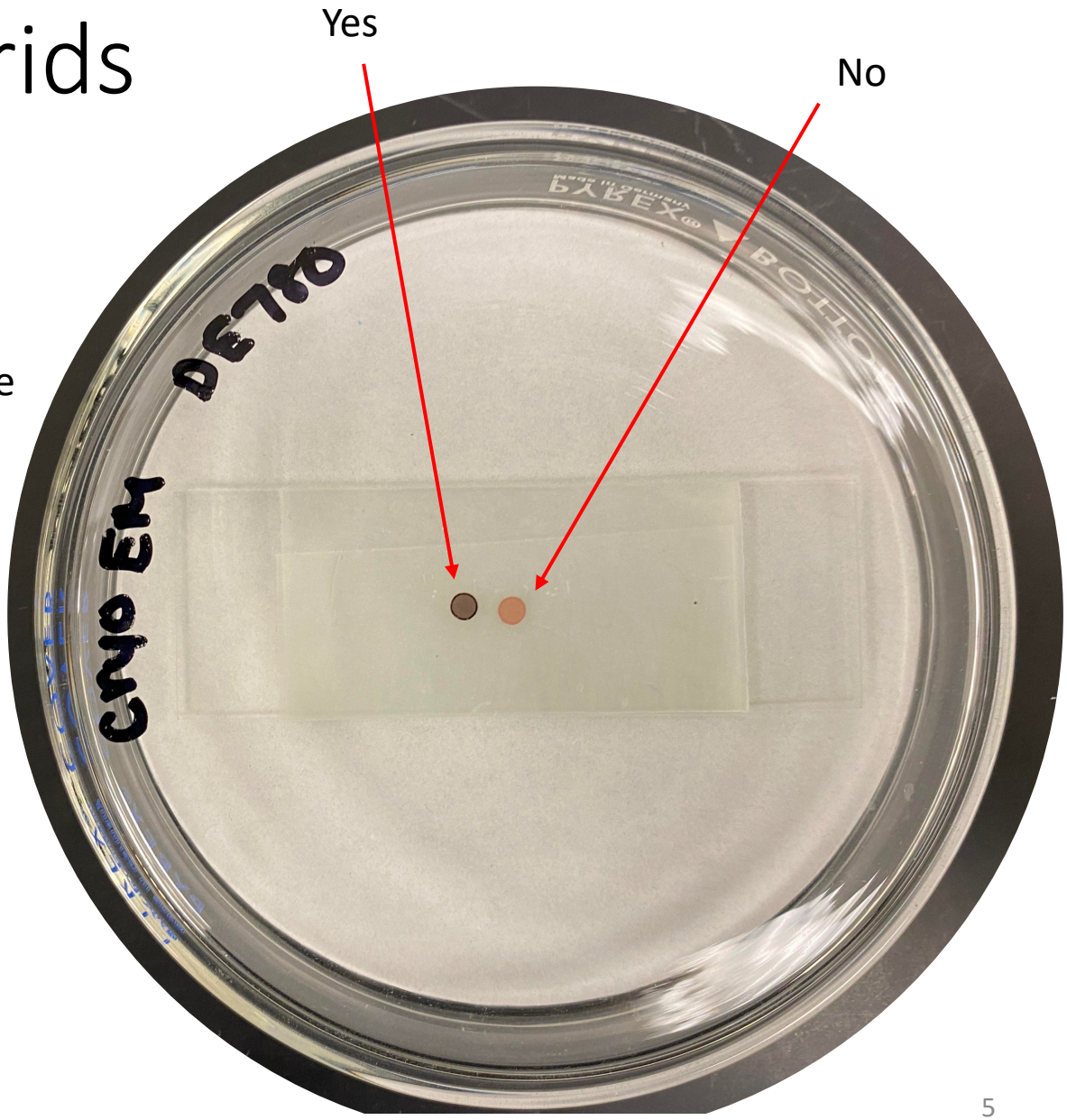
Options:

- Blot time: sec it blots
- Wait time: sec before blot
- Draintime: sec after blot
  - Usually zero!!
- Blot force: how tightly papers come together
- Blot total: # of blots
  - Usually 1!!

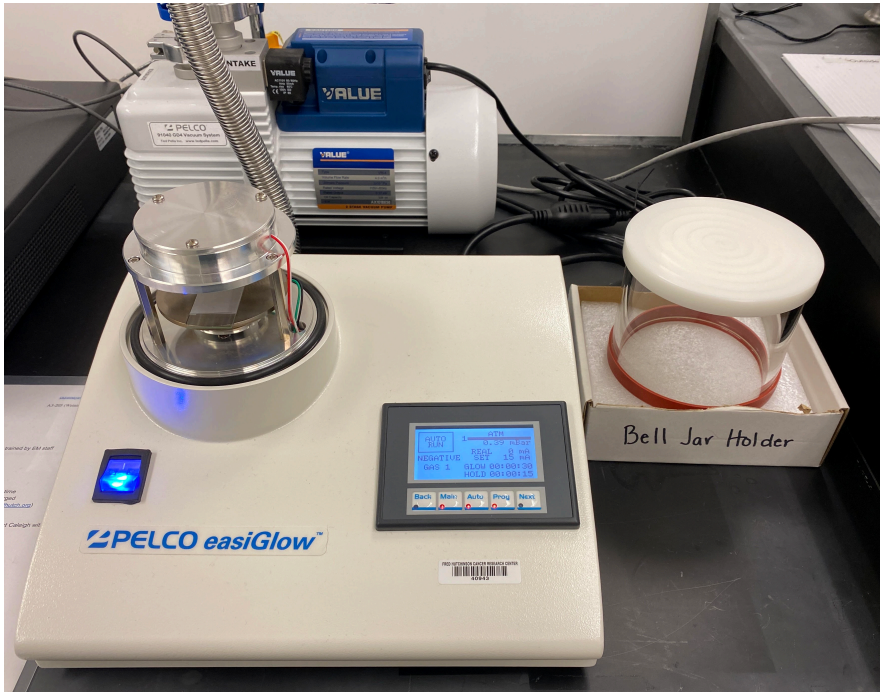
Record button label/sample etc on freezing log

Get your grids

- Lay out all the grids you want to stain in the whole session
- Carbon side up (dark/shiny)

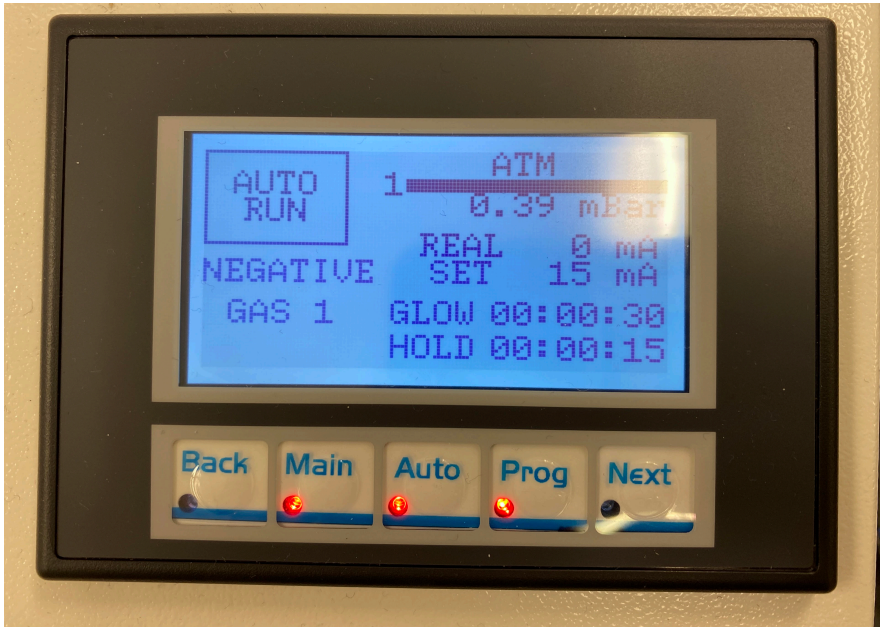


Glow discharge them



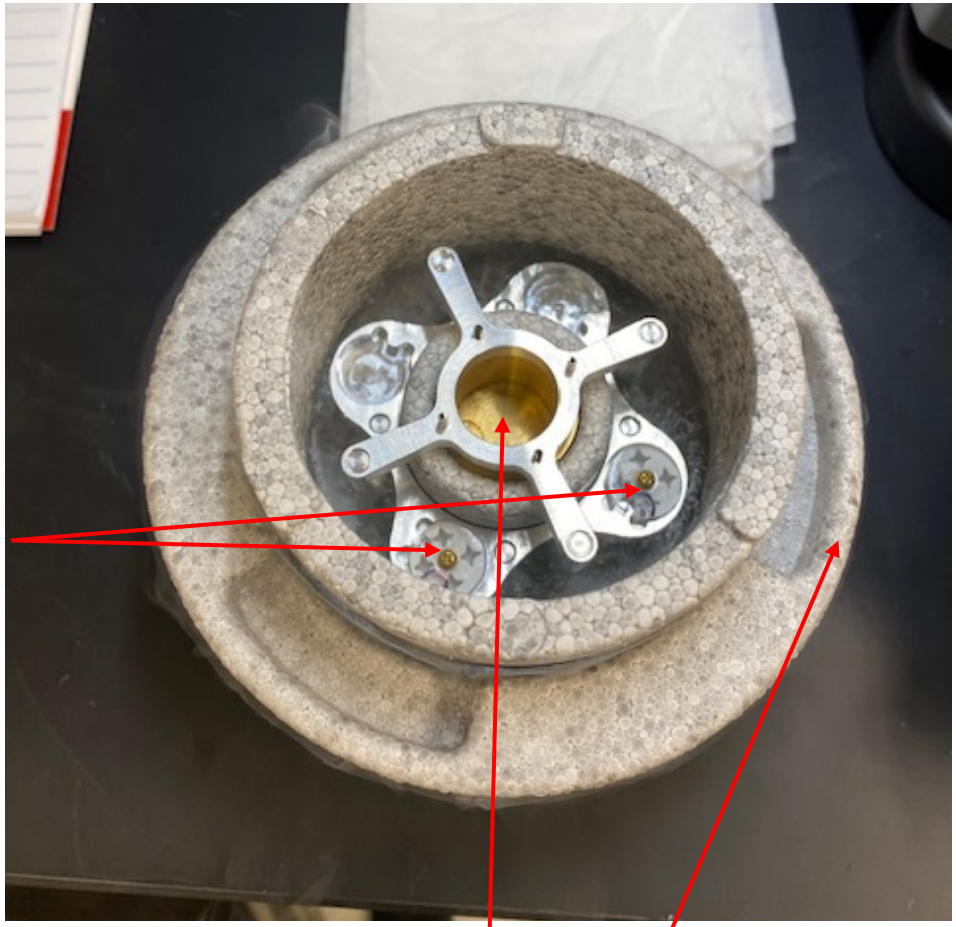
Standard settings should be fine

- 0.39 mbar
- Glow = 15mA
- Glow = 30s
- Hold = 15s



Turn on the easiGLOW and remove the bell jar carefully to insert slide with grids.

Cool freezing container

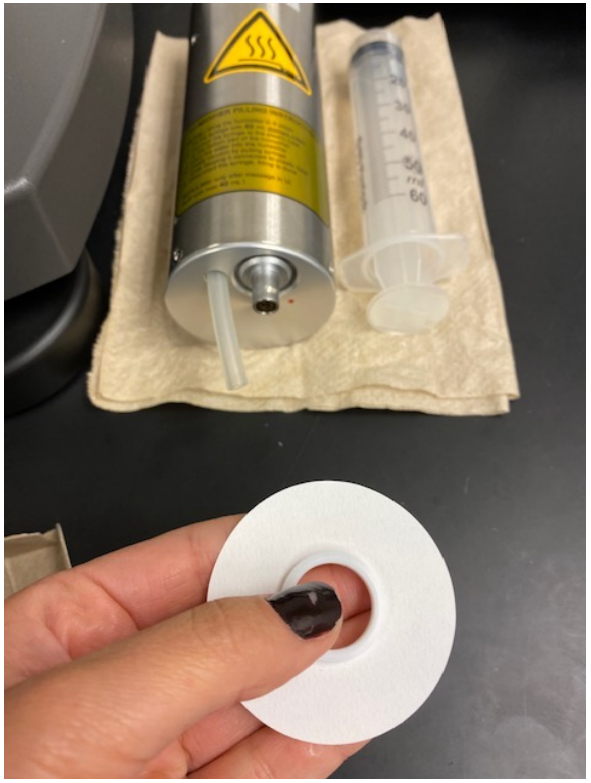


- Start when vitrobot is ~2-3deg above final temperature
- Buttons in holder counterclockwise (1)
- Fill LN2 in foam and ethane cup
- Sit for ~5min covered with foil top
- **No LN2 should remain in ethane cup** (2)
- Fill LN2 to top lip of foam container (3)

Attach blot papers to pads

Give filter papers 5~10 min in chamber before freezing

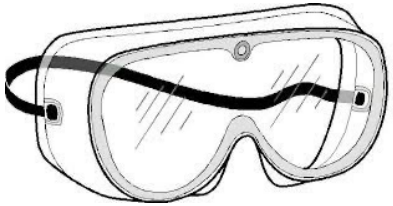
Remove center plastic ring



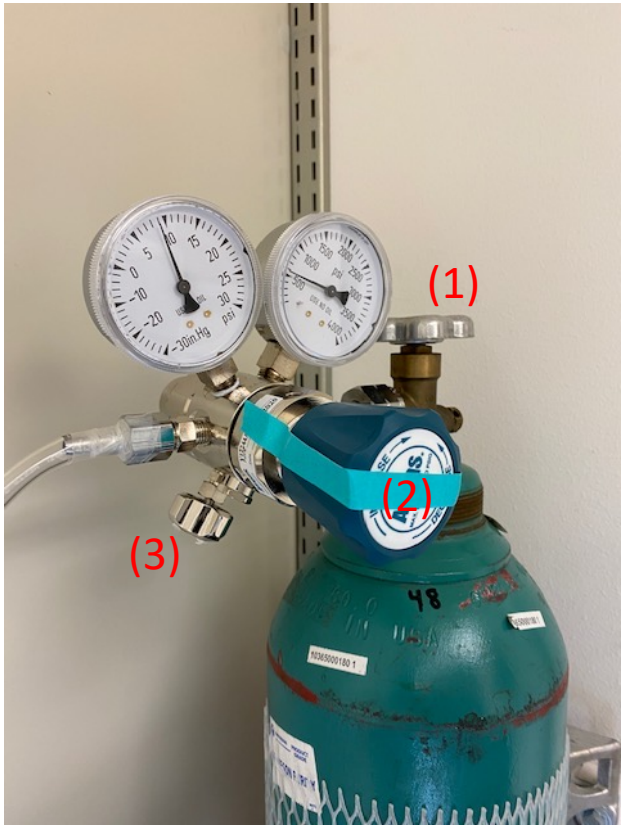
Attached to blot paper (use hole punch in drawer if needed) with curved side towards plastic

Affix to blot pad without squishing foam (should be easy to attached if well aligned)

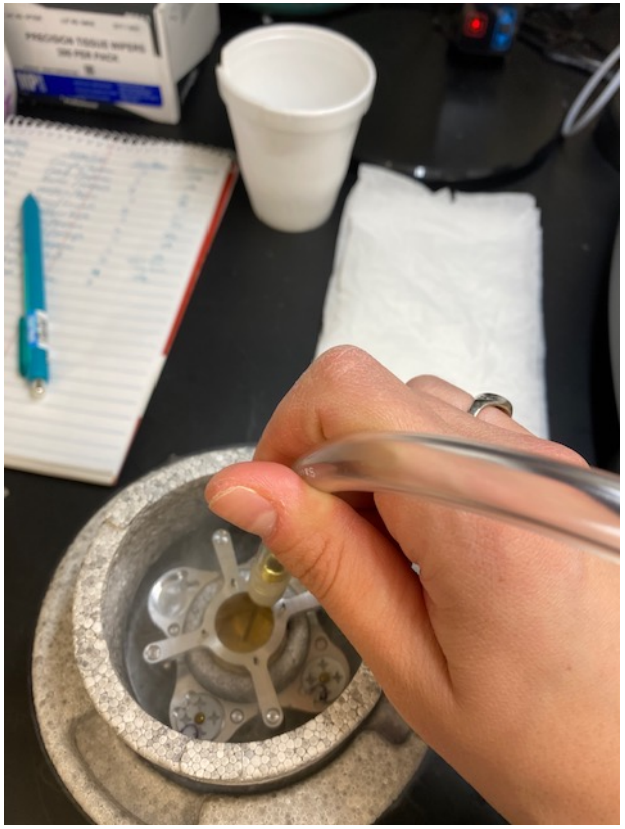
Fill ethane



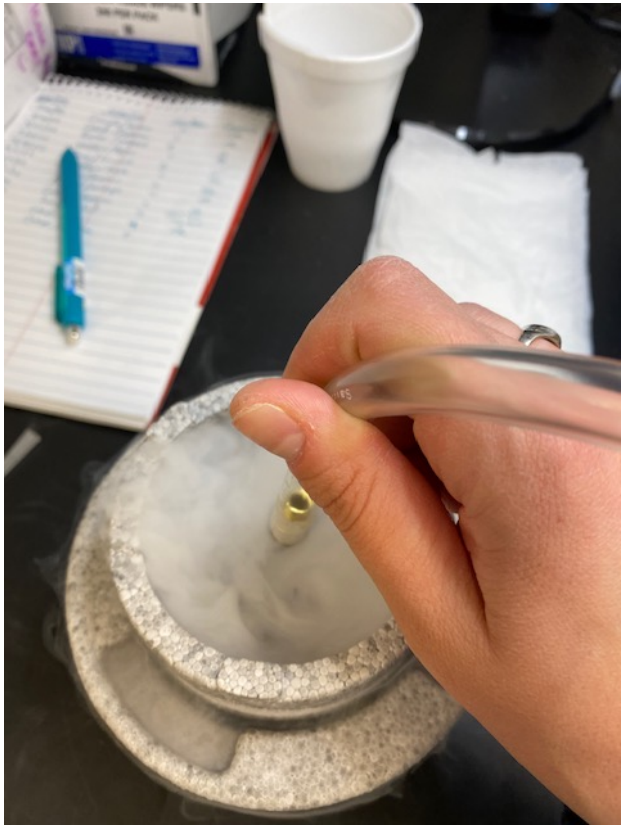
Goggles time!



(1) Open tank (counterclockwise)
(2) Do NOT touch middle regulator
(3) Open small knob (counterclockwise) to fill ethane cup

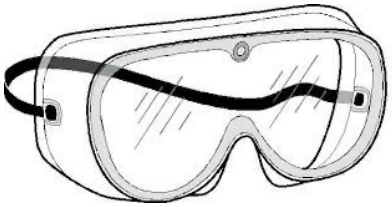


- Uncap ethane dispenser and remove from hood
- Place tip on bottom of ethane cup
- Open small knob (counterclockwise) gently and wait for “bacon frying” noise and fill to bottom of spider



- Do NOT lean tip onto spider
- Do NOT splash ethane

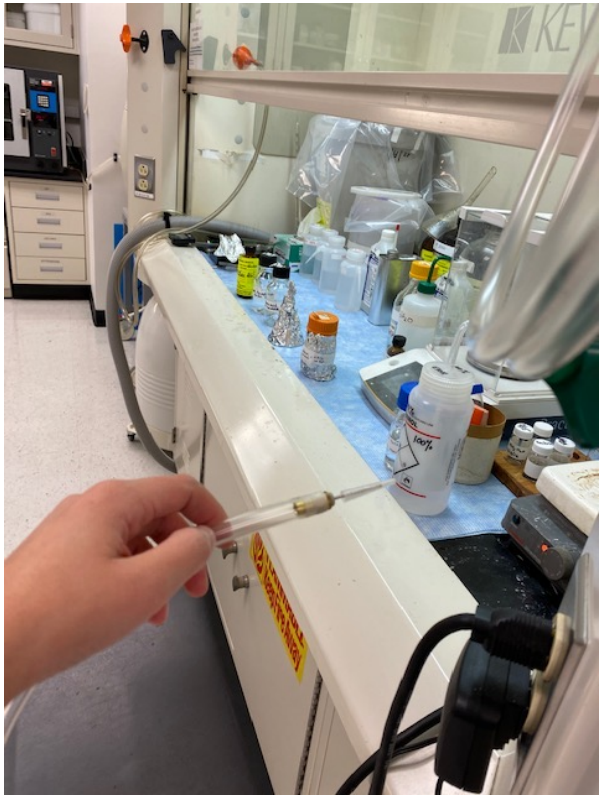
- DO wear safety goggles
- DO fill the ethane cup slowly
- DO lift foam ring up to make cup more visible



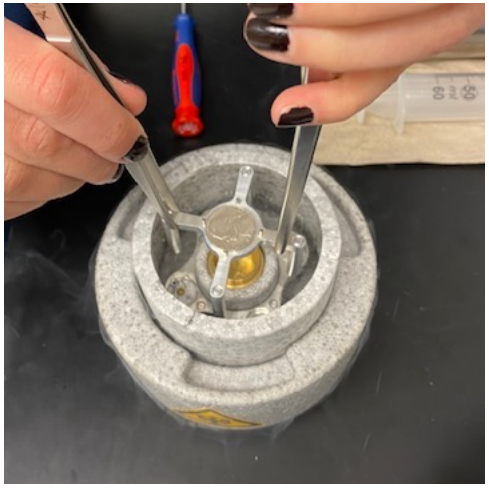
Goggles time!

Fill ethane

- Fill ethane to bottom of Spider – red line
- Fill LN2 to bottom lip of foam and wait until Spider freezes to ethane cup (can cover with foil)

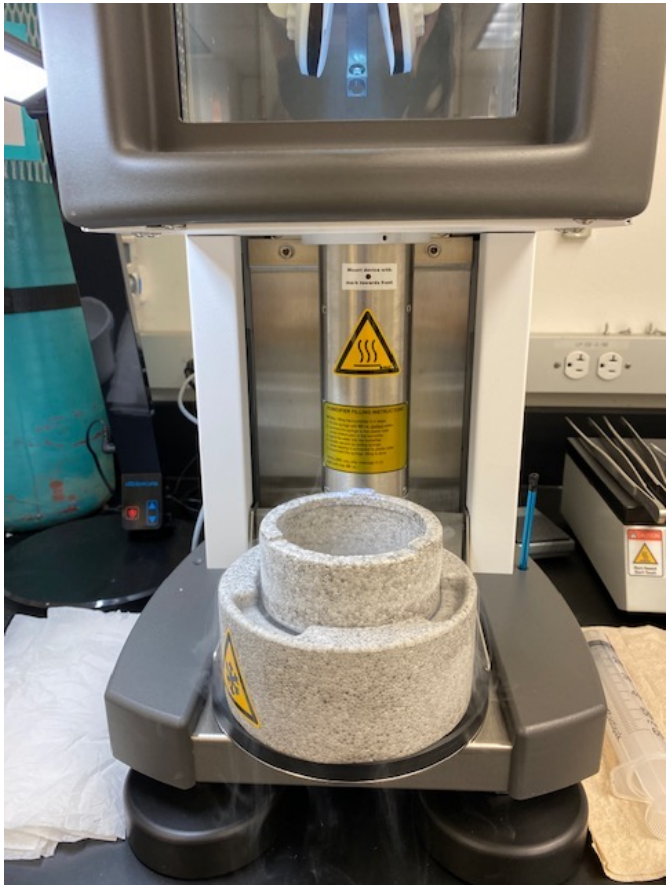


- Cap ethane tip (carefully!) to keep it clean and return to hood

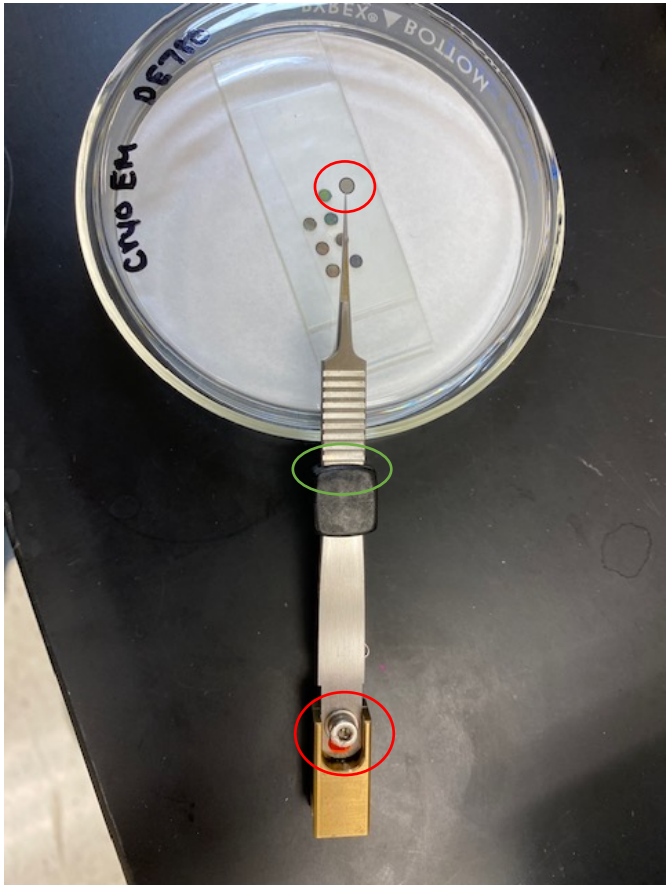


- Drop nickel between pins on top of spider
- Pick up opposite arms and lift straight up & out of freezing container

Setup for freezing

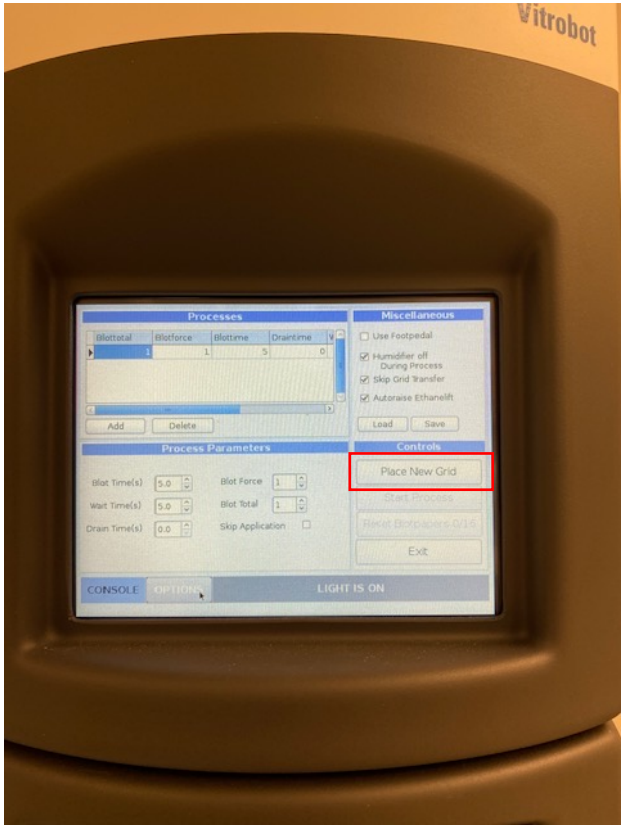


- Put freezing container with cold ethane on vitrobot (ethane must have some solids but not enough to hit grid)
- Uncover first slot in first grid



- Pick up grid, carbon side up, screw up (red)
- Slide clip down to first notch (green)

Place new grid

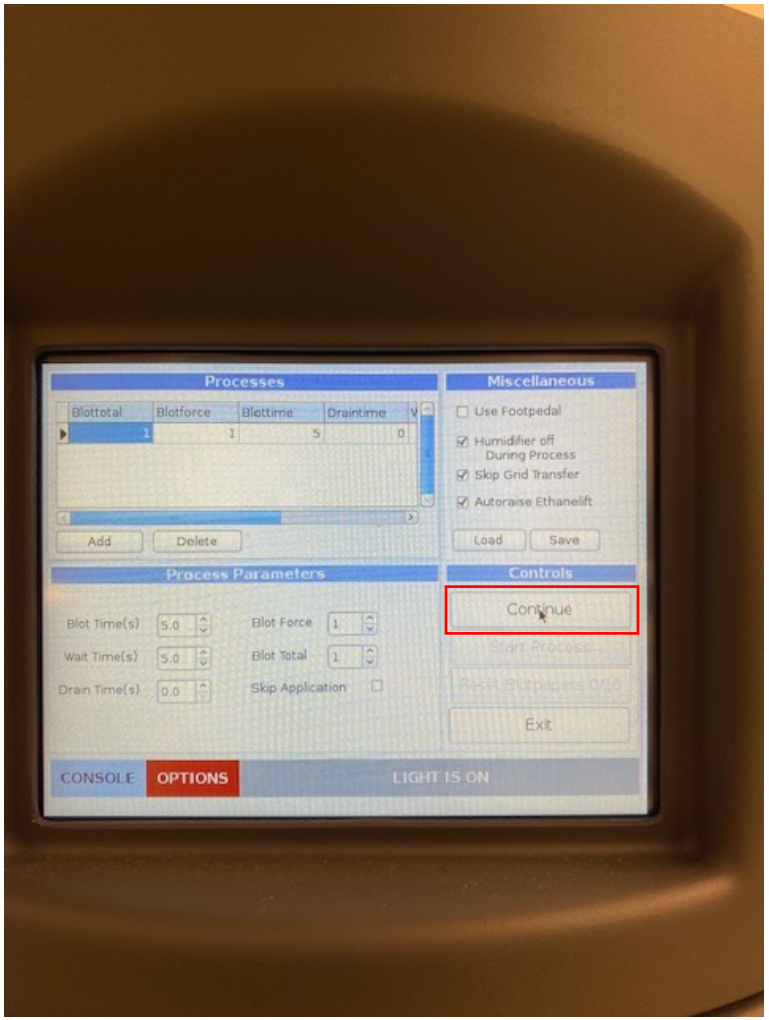


- Make sure Options are set for first blot
- Click – Place New Grid

- Tweezer holder raises up (green)
- Slide tweezers in at an angle with screw toward dominant hand

- Center tweezers on holder!
- Ethane here is good level of solid

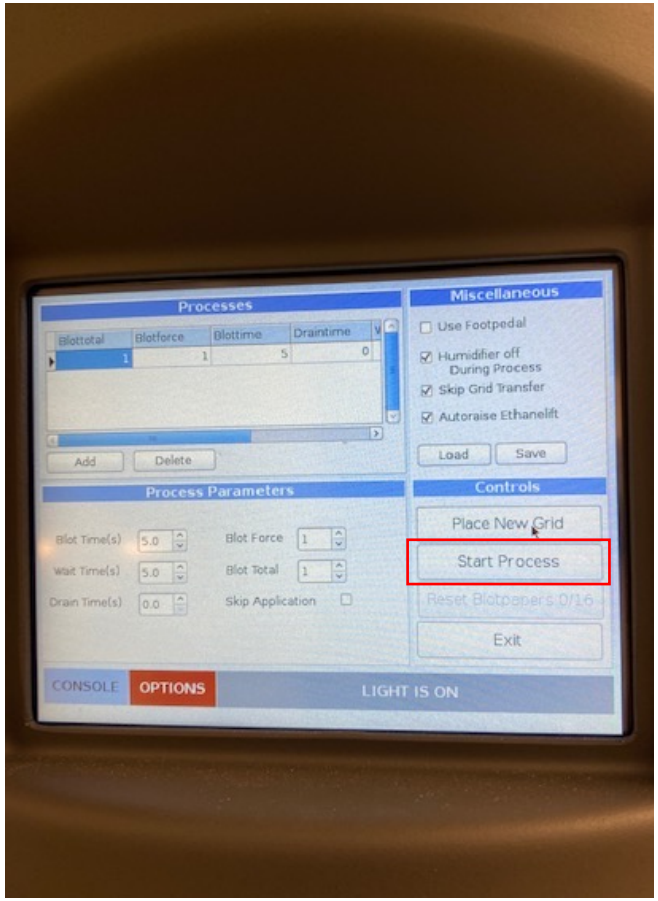
Continue



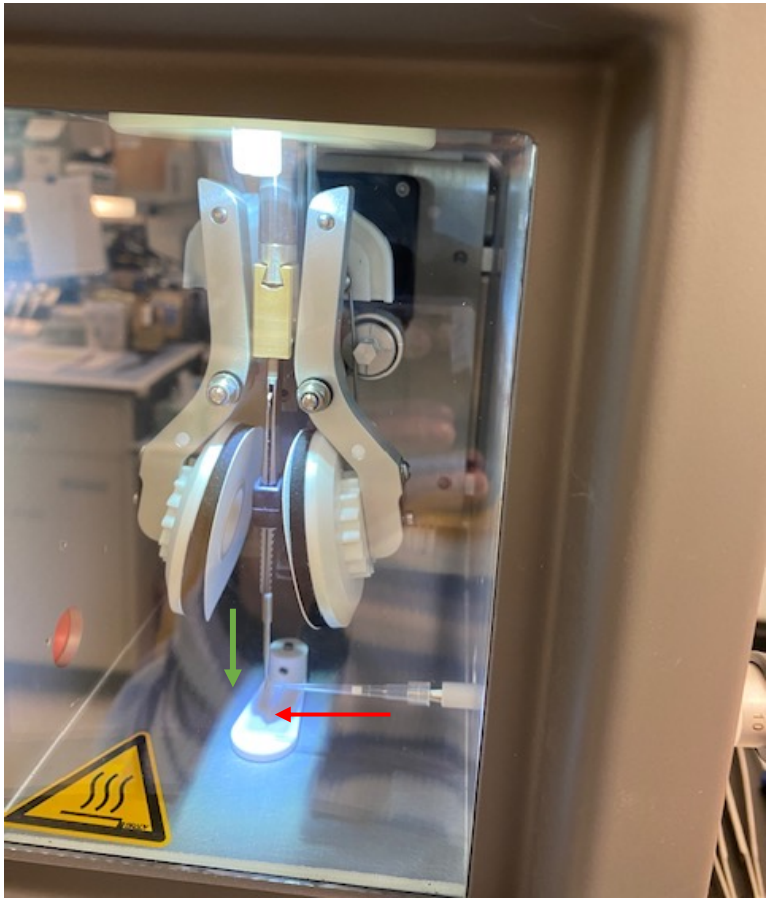
Place new grid turns into Continue

Click it to pull tweezers into chamber

Start process

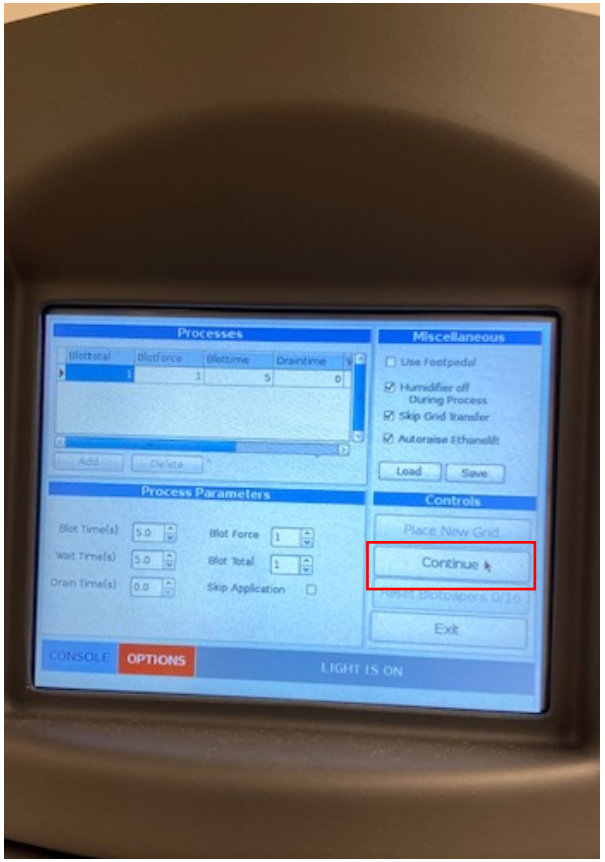


Click Start Process to bring grid down and apply sample



Tweezers drop below blot papers, apply 2-3uL of sample via opening on side

Continue

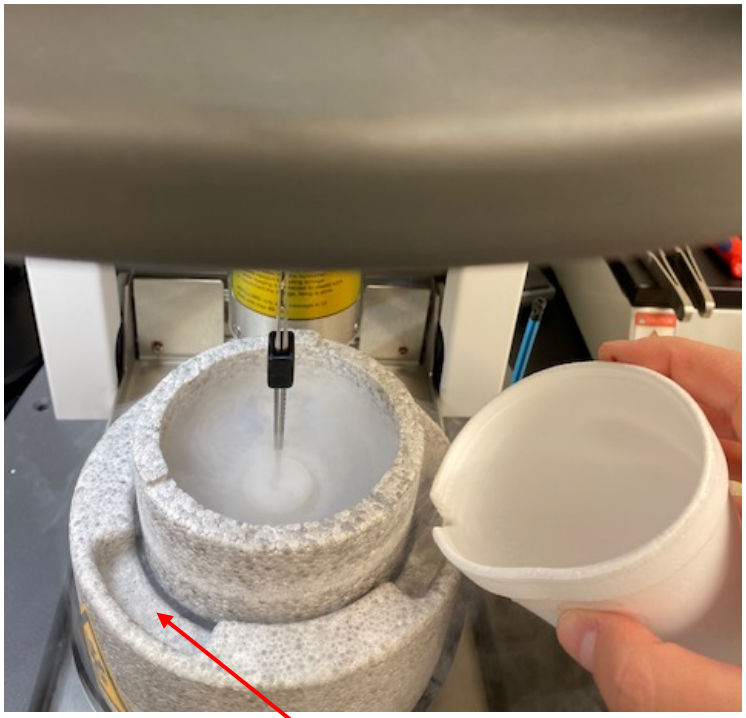


Start Process turns into
Continue
Click to freeze

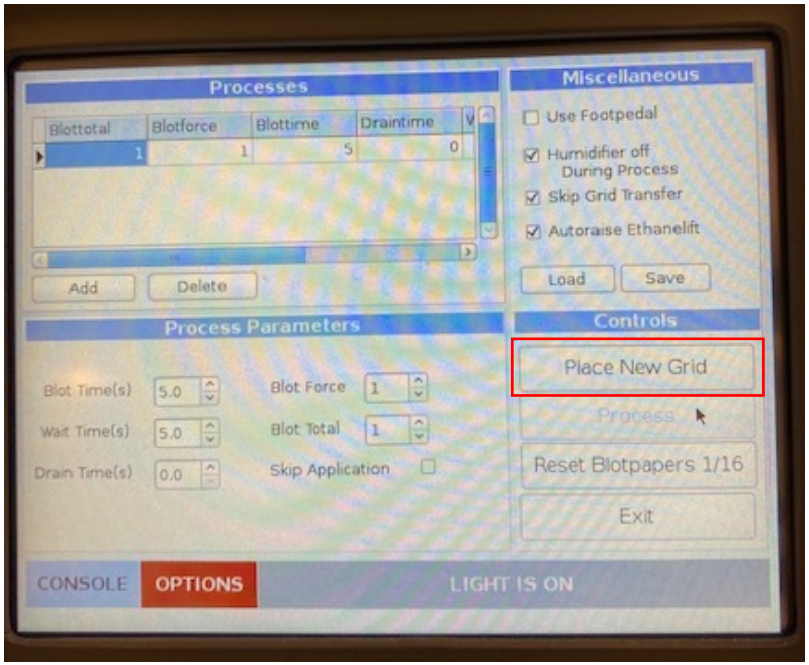
Pulls tweezers up (wait time)

Blots (blot time)
Pause before plunge (drain time)
Plunge freeze

Transfer grid to button and repeat until finished freezing



- Fill nitrogen to bottom lip
- Remove tweezers from vitrobot
- Hold tweezers closed and shift clip upwards
- Move grid to LN2
- Drop grid in correct button slot



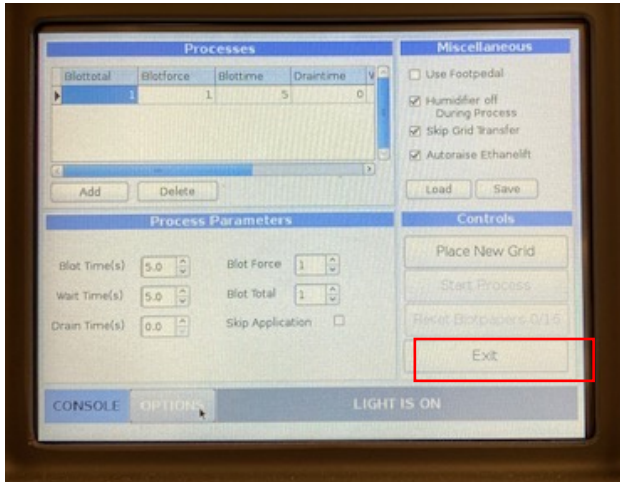
- Adjust options (usually blot time or force)
- Repeat from Place New Grid until finished freezing

Secure button(s) and store in puck/tube

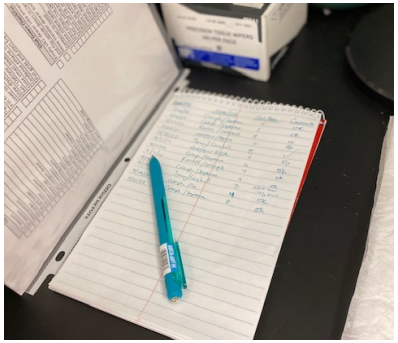
Use large tweezers to anchor lid so that it doesn't tighten with the notch over a grid slot

Drop button into deep nitrogen so you can get a good grip before transporting over to tube/puck

Cleanup

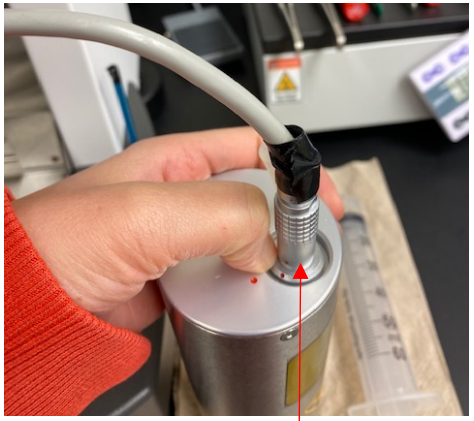


- Exit on vitrobot screen and allow computer to shutdown and THEN switch off on back



- Sign logbook

- Put freezing container in hood to evaporate



- Detach humidifier
- Reverse order of operations and then depress outer ring to release

- Store capped tweezers in vitrobot chamber for safe keeping
- Remove blot papers
- Store rest of tools on heatpad or drawer

- Turn off main ethane valve (clockwise)
- Vent remaining ethane into hood (small knob) and then close