Vitrobot Plunge Freezer Protocol:

Setup:
- Open nitrogen tank.
- Turn on Vitrobot at back right of machine.
- Replace blotting paper.
- Fill humidity cup to minimum fill line with picotap water and attach to humidifier.
- Enable foot pedal use if desired under Options menu on computer.
- Set blot time, set/enable humidity, and adjust any other parameters on computer.
- Cool cryo workstation with liquid nitrogen and use the "spider" to cool the ethane cup. Add ethane and **remove spider from workstation when ready to freeze**.
- Select "Place new grid" on menu or use foot pedal to attach Vitrobot tweezers with glow discharged grid.
- Place cryo workstation on Vitrobot **without the spider**.
- Re-enable humidity on computer when chamber is fully sealed.
- Add sample to grid at sampling loading position and close side opening.
- Plunge freeze.
- Transfer grid to grid box only after removing tweezers and cryo workstation from Vitrobot.

Shutdown Procedure:
- Raise plunge rod to uppermost position in chamber.
- Remove humidity cup, empty, and invert to dry.
- Remove blotting paper.
- Close nitrogen, ethane, and Solarus gas tanks.
- Shutdown Vitrobot computer.
- Turn off Vitrobot.
- Place cryo workstation in storage bin in lab.
- Return tools to where you found them and clean up any messes.