Safety

General Precautions:

- Please always be careful while working with liquid nitrogen (LN2).
- Use the Personal Protective Equipment (PPE) provided by PNCC: gloves, eye protection and cryo-gloves while working with liquid nitrogen.
- Liquid Nitrogen can cause severe frostbite.
- All PNCC microscope and wet lab rooms are equipped with oxygen monitors. In the event one of these units start alarming please evacuate the space you are in and meet the rest of the PNCC staff at the break room.

Liquid Nitrogen Use in the Wet Lab:

1. Closed toe shoes must be worn to enter the wet lab.
2. Before starting your wet lab work please make sure you are using the correct Personal Protective Equipment (PPE) (gloves, eye protection and cryo-gloves). If you wear glasses, please wear them while working with LN2.
3. If the tools in use get too cold, replace them with new ones to avoid freezing your fingertips. To reuse the tools, warm & dry them with the hair dryer and/or heat blocks available in the wet lab.
4. Do not refill 4L LN2 dewars by yourself. Ask on-site staff (SPOCs) to fill any dewars required for your work.
5. SPOCs will take care of the excess liquid nitrogen in the loading and clipping stations. Please do not discard of LN2 on the floor.

MANDATORY PPE
Setup

If your samples are BSL-2 stop immediately and notify PNCC staff

Only authorized PNCC staff may handle BSL-2 samples

- Verify that Ethane hose is not attached to Vitrojet. If attached, notify on-site PNCC staff.
- Select preferred ethane adapter: Standard Pipette Tip or Nanosoft Ethane Condenser.
  - For instructions, ask staff for assistance.
- Attach and fill humidifier with water available in yellow capped flask.
- Turn on tweezer warmer.
- Verify contents of “Freezing Kit” received from PNCC Staff:
  - Foam LN2 container
  - Vitrobot Tweezers
  - Blotting Paper & Pad Rings
  - Grids & Gridboxes
  - Freezing Cup
  - Pipettes & Tips
  - Two “Spiders”
  - Grid Storage Boxes
  - Filled 4L LN2 dewar

Shutdown

- Place ethane holder in fume hood to evaporate and dry.
- Detach humidifier from Vitrobot. Remove water and store upside down in Styrofoam box to the right of Vitrobot.
- Clean Vitrobot tweezers with EtOH, sheath tip.
- Notify staff that you have completed your vitrification session.
  Staff will store your grid box(es) in the PNCC Storage Dewar.