National Center for CryoEM Access and Training

Leica EM GP2 plunge freezer. NCCAT specific SOP additions.

Safety

General Precautions:

- Use the Personal Protective Equipment (PPE) provided by NCCAT. Nitrile gloves, goggles (or eyeglasses), and additionally cryo-gloves and a face shield while dispensing LN_2 and a face shield while dispensing ethane.
- The dehumidified room is equipped with an oxygen monitor. If the monitor alarm sounds, you **MUST** stop working, leave the room and leave the door propped open for air replacement. You must wait until air has recirculated and the alarm turns off before reentering the room (this generally takes only a minute or two).
    - <u>If you continue working in the room while the alarm is sounding, you will lose access to the lab.</u>
- Inspect equipment and accessories for weaknesses, cracks or damage before beginning work. If you suspect any damage, or encounter any problems during use, please fix immediately and ask for help from staff if needed.
- Report all accidents, no matter how minor.

Liquid Nitrogen:

- Ask for assistance to fill 4 L LN_2 dewars from the large tanks. Use cryo-gloves for LN_2 tank operation.
- Do not discard of LN_2 on the floor. Empty any used nitrogen into LD10 collection dewars.
- Be careful with any metal or tweezers in vacuum sealed dewars. If a dewar breaks, the release of vacuum can cause glass to shatter around the room.

Waste disposal

- Do not dispose of any chemicals or liquid cryogens down a sink drain. Liquid hazardous waste collection is in the SEMC fume hood. Pipette tips and other dry waste goes into red biohazard bags on the bench top or in metal bins under the bench. Gloves and masks must be disposed of in the hazardous waste collection.

Mandatory PPE

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

4.1 Setup (See Figure 1)

- Distilled water for the humidifier is kept in a 0.5 L bottle in the DH room (Figure 1).
- The ethane and nitrogen cups are in a small ice bucket in the fume chamber to the left of the GP2 (Figure 2). All other tools are in the silver case to the right of the GP2.
- The ethane tank is opened using two valves. Use valve 1 to control the flow rate and valve 2 to turn the flow on/off. Do not turn the valve covered in duct tape (Figure 3)
- The ethane condensing lid is on the left side of the GP2 and already connected to the ethane tank.

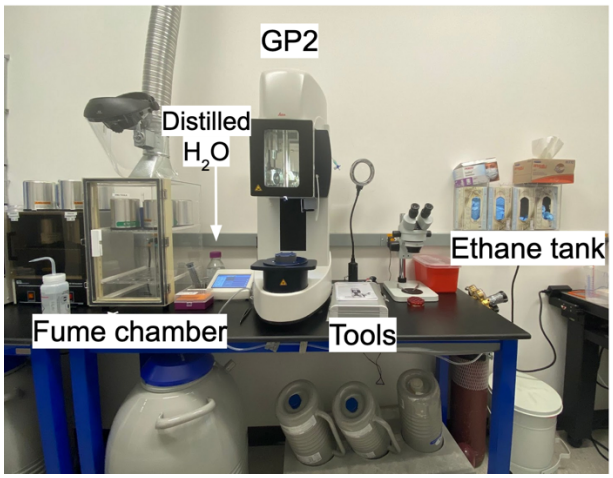

Figure 1. GP2 setup in the NCCAT DH room

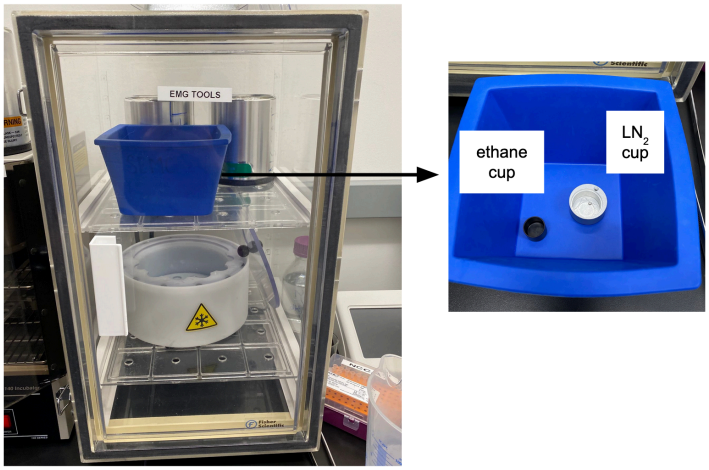

Figure 2. Cryogen cups are kept in the fume chamber

4.1.11 Standard blotter settings:

Horizontal blot position: 42.0 mm
Vertical blot position: 3.0 mm

4.3 Shutdown

- Place ethane cup and nitrogen cup with any remaining ethane and liquid nitrogen in the blue bin inside the fume chamber to evaporate and dry (figure 2).
- Close both ethane tank valves (1 and 2 - figure 3).
- Clean tweezers with water and ethanol and return to the silver box.
- Empty the LD4 of any unused nitrogen into an LD10 collection dewar and invert on a rack to dry.
- Return all tools into the drawers and leave the bench top empty and clean.

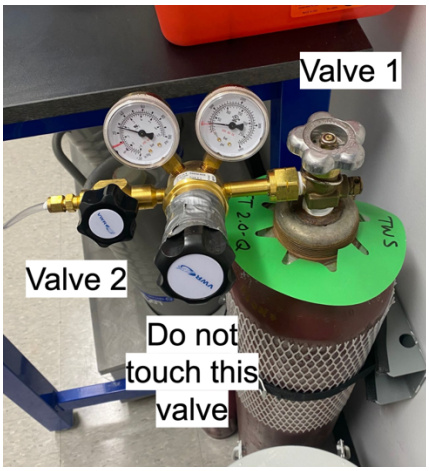

Figure 3. Ethane flow is controlled using valves 1 and 2. Adjust dispensing rate with valve 1.

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