



Cryo-EM Merit Badge Standard Operating Procedure

A Collaboration Among the NIH-Funded Cryo-EM National Centers

Common Leica GP2 Plunge Freezer Standard Operating Procedure

version 1.1

Authors: Htet Khant, Sean Mulligan, Christina Zimanyi

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1. Purpose

- 1.1. Setting up, operating, and shutting down the Leica EM-GP2 in a safe and effective manner for the preparation of frozen cryo-EM of samples. Note: This SOP is for BSL-1 work only.

2. Definitions:

- 2.1. Leica GP2 is a vitrification device for freezing specimens to use for cryoEM analysis.
- 2.2. Liquid Nitrogen (LN2) is a cryogenic liquid stored under pressure.
- 2.3. Ethane is classified as a flammable gas.

3. Supplies & Equipment

- ☐ PPE (BSL-1)***(also see site specific instructions)**
 - ☐ Laboratory Coat
 - ☐ Nitrile Gloves
 - ☐ Goggles / Safety Glasses
 - ☐ Cryogenic Gloves
 - ☐ Face Shield
- ☐ Chemicals/Reagents
 - ☐ Liquid Nitrogen
 - ☐ Ethane
 - ☐ 70% Ethanol
 - ☐ Distilled Water
 - ☐ Sample
- ☐ Filter Paper (Pre-punched Whatman #1 or #541)
- ☐ Pipette and Tips
- ☐ Tweezers for misc. grid handling
- ☐ Leica GP2 Tweezers
- ☐ Glow Discharged / Plasma Cleaned EM Grids
- ☐ Grid Storage Boxes/Buttons
- ☐ Grid Box Lid Opening Tool (Screwdriver or TFS Pin Tool)
- ☐ LN2 Dewars for Transfer
- ☐ Forceps (fine tip and long tweezers)

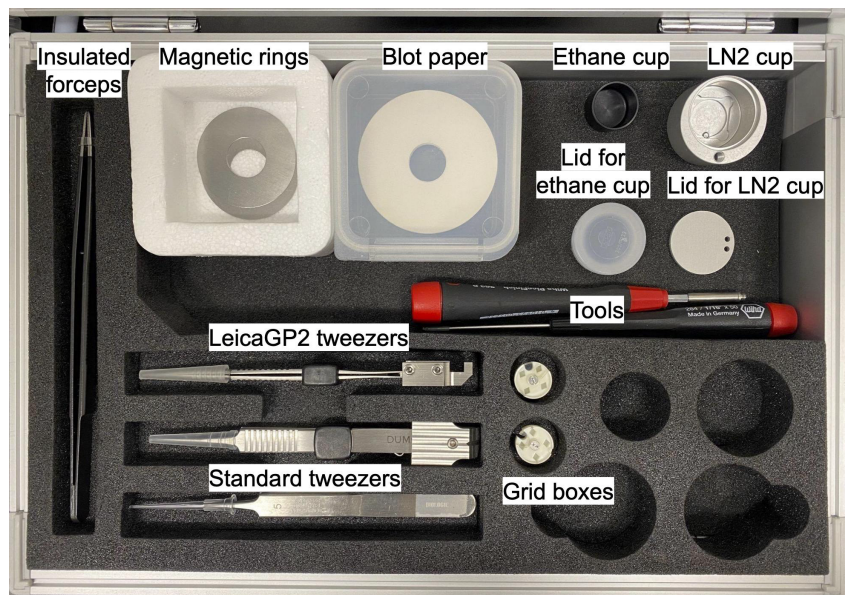


Figure 1. Leica EM GP2 tools

4. Procedure:

- 4.1. Instrument setup and cryogen preparation (takes 30-60 min) ***(also see site specific instructions for 4.1)**
 - 4.1.1. Turn on the unit using the switch on the back bottom right of the main unit. Following the prompts on the touch screen:
 - 4.1.2. Place a fresh filter paper on the blotting arm, using the metal ring to secure it well centered on the magnetic mount. Press “OK” on the touch screen. (Filter paper should be in the chamber about 15-20 min. before you blot any grids).



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4.1.3. Using a syringe, fill the humidifier with distilled water until the warning prompt turns off (usually ~60 mL) through the fill/drain hose. Once you have added the 60 mL the touch screen warning will turn off. Turn the full/drain hose connection to closed and hang it on it's storage clip

4.1.4. Check the drip/drain tray and empty if needed.

4.1.5. Place the black cryogen cup and silver transfer container, with your grid box, into their spots in the freezing chamber.

4.1.6. Pour LN₂ into the freezing chamber until it is 100% filled (this will take ~1.8 L of LN₂. Refill as needed during the freezing session. The LN₂ level should remain below the metal mesh while the cryogen and transfer containers remain cold in a nitrogen vapor environment.

4.1.7. Once the cold chamber temperature has reached -175°C, use the ethane fill lid to condense ethane into the black cryogen cup.

4.1.8. Add LN₂ as needed while you're working. Once you have added ethane to the cryogen cup, be careful not to spill LN₂ into it while topping off the cold chamber or silver transfer cup. You can use the cryogen container cover to protect it if you need.

4.1.9. Set up the environmental chamber settings. Typical settings:

Temperature	4-25°C
Relative Humidity	0-99%
Cryogen Temperature	-175°C
Cryogen GN2 flow	100%

Note: humidifier will only run when chamber temperature is reached

4.1.10. Set up the Load Specimen Parameters, enable rotation of the forceps during blotting if desired.

4.1.11. Set up the blotting parameters

Typical blot times are 0.5-3.0 s.

Adjust Horizontal and Vertical blot positions: load a blank grid on the tweezers then load the tweezers onto the plunge rod. Next, click the "Set" button. The chamber will lower to blotting position and you can adjust the tweezer position using the control panel. You can use the binoculars if available. *(see site specific details for typical settings).

4.1.12. Set up the Plunge/Transfer parameters

4.1.13. **Make sure all cryogen containers in the freezing chamber are uncovered** then test your settings by running through a blotting cycle with the tweezers attached. Repeat from 4.1.10 if needed.

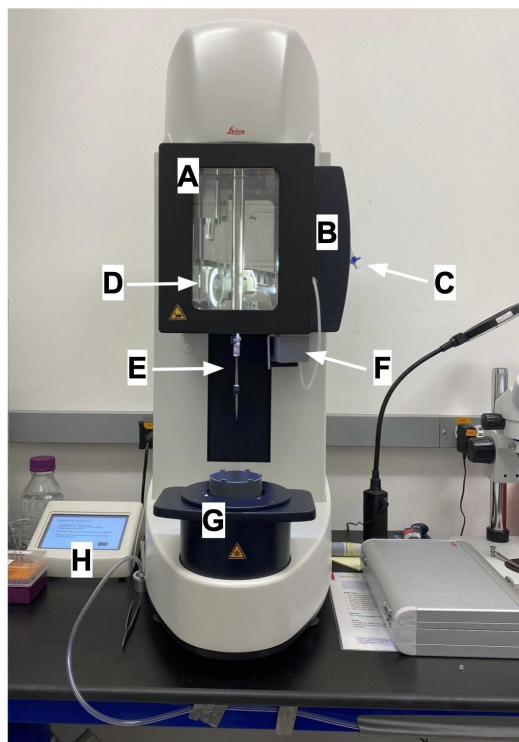


Figure 2. Leica EM GP2 instrument components

- A) Environmental chamber
- B) Humidifier tank
- C) Humidifier hose valve
- D) Blot paper attached with ring magnet
- E) Tweezer attached to plunge rod
- F) Drip Tray
- G) Freezing station
- H) Touch screen/instrument control



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4.2. Freezing Cycle / Plunge Freezing

- 4.2.1. If you didn't attach blot papers to the blotting pads in step 4.1, do so now. Replace blot papers after every 10 blots or every 20 minutes, whichever occurs first. Optional: Reset the Leica GP2's counter so the system will keep track for you.
- 4.2.2. Make sure you have a grid box in the silver transfer container, with an open slot ready to transfer your grid into.
- 4.2.3. Pick up a glow discharged grid with the Leica GP2 tweezers. Please note, the tweezers can only be attached to the plunge rod in one orientation. Be mindful of which side your foil faces, so that you can apply your sample to the side you want (the system allows you to rotate the tweezers during the plunging protocol if incorrect).
- 4.2.4. Proceed to the "LOAD FORCEPS" position. After the environmental chamber has completely lifted, attach the tweezers holding your grid. Tweezers will "click" into place when properly attached to the plunge rod.
- 4.2.5. **Make sure all cryogen containers in the freezing chamber are uncovered**, then lower the chamber to blotting position by pressing the "LOAD SPECIMEN" button.
- 4.2.6. Apply your sample to the grid using the chamber side ports. Keep the side ports closed when not actively pipetting to maintain the chamber environment.
- 4.2.7. Press "BLOT" to start the blotting process. If you did not enable automatic plunging, the "PLUNGE" button will need to be pressed when it becomes available. If you did set up automatic plunging, the system will plunge the grid into liquid ethane after blotting.
- 4.2.8. After plunging, the chamber will automatically raise.
- 4.2.9. Now you must place your vitrified grid into the gridbox. Remove the tweezers from the plunge rod and transfer your grid into an open slot in your grid box. The gridbox is in the silver transfer container and you will need to quickly move between the ethane cup and transfer container.
- 4.2.10. Repeat steps 4.2.3 to 4.2.9 until all your grids are vitrified.

4.3. Shutdown *(also see site specific instructions for 4.3)

- 4.3.1. Clean, dry and store the Leica GP2 tweezers.
- 4.3.2. Empty the humidifier by aspirating from the fill tubing with the empty 60 mL syringe.
- 4.3.3. Empty the drip tray.
- 4.3.4. Remove and dispose of blot paper and store the magnetic ring.
- 4.3.5. Remove both cryogen containers and put in appropriate location (see site specific instructions)
- 4.3.6. Follow the touchpad instructions to start the bake out, with a bake out time of 1 hour (this will take 1-2 hours).
- 4.3.7. Once the bake out is completed power off the instrument.

5. Chemicals:

- 5.1. Ethane
- 5.2. Liquid Nitrogen
- 5.3. Ethanol 70%

6. Waste Disposal:

- 6.1. Follow facility procedure for proper disposal (see site specific instructions).
- 6.2. Biohazardous waste will be collected in designated bins lined with red biohazard bags.
- 6.3. Chemical hazardous waste will be segregated by hazard class (e.g. flammable, corrosive) and state (e.g. solid, liquid), appropriately labelled, and placed in the laboratory's hazardous waste collection.