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**Purpose:** To setup a Gatan CP3 Plunge Freezer.

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**Approved:**

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**1. Purpose:**

- 1.1. To setup a Leica EM-GP for operation and shutdown.

**2. Scope:**

- 2.1. To safely setup a Leica EM-GP and to shut it down.

**3. Definitions**

- 3.1. Shutdown of a Leica EM-GP for the safety of the scientific device and considerations for the next user.
- 3.2. Setup of a Leica EM-GP for the safe operation of the scientific device.

**4. Responsibilities:**

- 4.1. Turn the machine on (power switch to the back-bottom right of the machine).
  - 4.1.1. Setup of the Leica EM-GP takes 30 - 60 minutes.
- 4.2. Place the black ethane cup and the silver transfer container into the Dewar chamber (should be stored inside of a fume hood).
- 4.3. Using a syringe, fill the humidifier with 60 mL of distilled water.
- 4.4. Fill the Dewar chamber with LN<sub>2</sub> until 100% full. Refill as needed.
- 4.5. Cautiously transfer your labeled cryo grid box into the silver transfer container.
- 4.6. Once the cryogen container temperature reaches less than -175°C, liquefy ethane gas in the black container until it is full. Carefully fill the silver transfer container with liquid nitrogen. Refill as needed.
- 4.7. Setup environmental chamber settings.
  - 4.7.1. T<sub>C</sub> = 4°C - 25°C
  - 4.7.2. H<sub>R</sub> = 90 - 99%;
  - 4.7.3. NOTE- humidifier will only run when TC is reached
  - 4.7.4. Setup temperature of cryogen = -175°C
- 4.8. Setup blotting parameters refer to figure 1.
  - 4.8.1. On the main screen, click the long rectangular gray box in the upper left side (P1 \_\_\_\_/1.0/\_\_\_\_).
  - 4.8.2. Select a Nr. and edit the parameters as needed.
  - 4.8.3. Select "OK" to exit screen.
  - 4.8.4. Setup tweezer/blotter position parameters. The 2 main settings that need to be adjusted are "blotter settings" and "grid blot position".
  - 4.8.5. On the main screen, select "Setup" button.
- 4.9. Blotter settings
- 4.10. Click "Adjust"
- 4.11. Lower chamber, OK
- 4.12. Select "Blot Position"
  - 4.12.1. Using binoculars and adjust buttons, move blotter closer or further from grid to optimize contact (218 suggested)
  - 4.12.2. Select "Back" when done



- 4.13. Grid Blot Position
  - 4.13.1. Click “Blot Position”
  - 4.13.2. Using microscope and adjust buttons, move tweezer up or down from blotter to optimize contact (3.6mm suggested).
  - 4.13.3. Select “Back” when done
- 4.14. Select “Prepare” to exit screen
- 4.15. Freezing grids
  - 4.15.1. Add new blotting paper and secure using the magnetic ring. Mark blotting paper with pencil to track rotation of paper. Switch blotting paper every 4 grids.
  - 4.15.2. Pick up your grid, carbon side facing the blotting paper, with the forceps (refer to figure 2).
  - 4.15.3. Select “Lower chamber/R”
  - 4.15.4. Add your sample to right side of grid
  - 4.15.5. Leica EM-GP will incubate for your allotted time selected in settings
  - 4.15.6. Select “Rotate home, Blot/A-Plunge”
  - 4.15.7. Remove tweezers from the ethane cup and quickly and carefully transfer your frozen grid to your cryo gridbox.
  - 4.15.8. Repeat 4.15.2 - 4.15.7 until done.
- 4.16. If you are the last user for the day, then continue to step below. If not, then top off nitrogen in Dewar, cover nitrogen Dewar with a Kim Tech wipe (to prevent frosting) and empty the water from the humidifier (to prevent buildup of water condensation in the chamber).
- 4.17. Remove forceps, wipe them down with ethanol and put in storage.
- 4.18. Empty water from humidifier.
- 4.19. Open environmental chamber door.
- 4.20. Discard blotting paper and store magnetic ring.
- 4.21. Remove both cryogen containers and transfer to fume hood.
- 4.22. Click “Bake Out” for 1 hour (default).
  - 4.22.1. This takes ~ 2 hours total.
- 4.23. When bakeout is complete, lower the chamber and turn the machine off.
- 4.24. Cleanup will look like Figure 3

## **5. Personal protective Equipment (PPE):**

- 5.1. Laboratory coat
- 5.2. Nitrile gloves
- 5.3. Goggles
- 5.4. Cryogenic gloves
- 5.5. Face mask

## **6. Chemicals:**

- 6.1. Ethanol 70%

## **7. Equipment**

- 7.1. Liquid Nitrogen (LD4 or LD5)
- 7.2. Tweezers
- 7.3. Small Screwdriver or Gripper Tool for your cryo grid box
- 7.4. Cryo Grid Box
- 7.5. Transfer Dewar



- 7.6. Pipette
- 7.7. Pipette tips
- 7.8. ddH<sub>2</sub>O
- 7.9. Sample
- 7.10. Plasma Cleaned Grids
- 7.11. Filter Paper

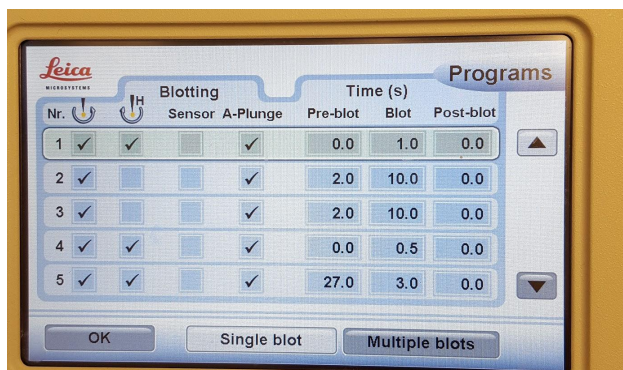
**8. Waste Disposal:**

- 8.1. N/A

**9. Vendors:**

- 9.1. N/A

**Figure 1**



**Figure 2**

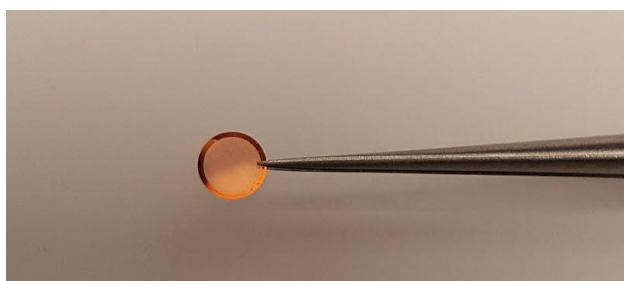




Figure 3

