



Purpose: To setup a Gatan CP3 Plunge Freezer.

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Approved:

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1. Purpose:

- 1.1. To setup a Gatan CP3 in an effective and safe manner.

2. Scope:

- 2.1. To safely setup a Gatan CP3 and to shut it down.

3. Definitions

- 3.1. Shutdown of a Gatan CP3 for the safety of the scientific device and considerations for the next user.
- 3.2. Setup of a Gatan CP3 for the safe operation of the scientific device.

4. Responsibilities:

- 4.1. Turn on the power switch on the right side.
- 4.2. Open the nitrogen tank.
- 4.3. Test plunger without a sample first by making sure the safety shield is in place and pressing 'reset', then 'start'. If there are any issues, please contact a member of EMG.
- 4.4. Moisten sponge in hot water then insert sponge into chamber.
 - 4.4.1. You may need to moisten the sponge several times. Ideally, you want the relative humidity to reach 80-90% so as not to dry out your sample.
- 4.5. Add fresh, uncontaminated liquid nitrogen to the workstation.
- 4.6. Gently pull blotters out to remove.
- 4.7. Replace filter paper before using plunger.
 - 4.7.1. You are not expected to replace it after use, so it is safe to assume the filter paper is contaminated with another sample.
 - 4.7.2. Use tweezers to remove the pin and release used filter paper and plastic disk.
 - 4.7.2.1. Take care not to lose the plastic disk!
 - 4.7.3. Place inverted pin in the bottom of the filter paper holder, followed by new filter paper and plastic disk.
 - 4.7.4. Push down gently with blotter to attach.
 - 4.7.4.1. If you need to make more filter paper disks, please see a staff member.
- 4.8. Condense 5mL ethane in a liquid nitrogen-cooled 50mL conical tube.
- 4.9. Set blotting time.
- 4.10. When set temperature (-174°C) and relative humidity is reached, insert the cryo plunge freezer tweezers with plasma cleaned grid into plunge rod.
- 4.11. Add 2-3µl of sample to grid using pipette.
- 4.12. Rotate plunge rod 90 degrees.
- 4.13. Close the safety shield, press 'reset' then 'start' to blot sample and plunge into liquid ethane.
- 4.14. Open the safety shield and raise the plunge rod just enough to remove tweezers by pressing the blue button but not remove the grid from the ethane pool.
- 4.15. Quickly transfer grid into liquid nitrogen and into cryo grid box.
- 4.16. Lift plunge rod before turning off Cp3!
- 4.17. Close nitrogen and ethane tanks.



- 4.17.1. Make sure that plasma cleaner gas tanks are also closed.
- 4.18. Remove the sponge from the chamber.
- 4.19. Return tools to designated locations and clean up any messes.

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. ddH₂O
- 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