

# Cp3 Plunge Freezer Protocol:

## Setup:

- Turn on power switch on right side.
- Open nitrogen tank.
- Test plunger without a sample first by making sure safety shield is in place and pressing 'reset', then 'start'. If there are any problems please contact Ashleigh or another member of EMG.
- Insert sponge/humidity wand into chamber after wetting with hot water. You may need to re-wet the sponge several times. Ideally you want the relative humidity to reach 80-90% so as not to dry out your sample.
- Add fresh, uncontaminated liquid nitrogen to the workstation.
- Replace filter paper before using plunger. You are not expected to replace after use so it is safe to assume the filter paper is contaminated with another sample.
  - Gently pull out blotters to remove.
  - Use tweezers to remove pin and release used filter paper and plastic disk. Take care not to lose the plastic disk! Place inverted pin in bottom of filter paper loading station followed by new filter paper and plastic disk. Push down gently with blotter to attach. If you need to make more filter paper disks please see a staff member.
- Condense 5mL ethane in a liquid nitrogen-cooled 50mL conical tube. Be sure to wear protective gear and make sure to close both valves on ethane when finished.
- Set blotting time.
- When set temperature (-174°C) and relative humidity has been reached, insert cryo plunge freezer tweezers with plasma cleaned grid into plunge rod.
- Add 2-3µl of sample to grid using pipette. Rotate plunge rod 90 degrees.
- Close safety shield, press 'reset' then 'start' to blot sample and plunge into liquid ethane.
- Open safety shield and raise plunge rod just enough to remove tweezers by pressing blue button but not remove grid from ethane pool. Quickly transfer grid into liquid nitrogen and into cryo grid box.

## Shutdown Procedure:

- **Lift plunge rod before turning off Cp3!**
- Turn off nitrogen and ethane.
- Make sure plasma cleaner gas tanks are also closed.
- Remove sponge wand from chamber.
- Return tools to where you found them and clean up any messes.

For more advanced instructions, you can find the Cp3 manual here:

<http://www.nysbc.net/twiki/pub/Main/PlungeFreezing/CP3PlungeFreezerManual.pdf>