



## CP3 Cryoplunge Operation Checklist

### Pre-Cooling Setup

- Ensure liquid nitrogen (LN<sub>2</sub>) dewar is clean and dry.
  - Fill the CP3 LN<sub>2</sub> container with fresh liquid nitrogen.
  - Chill the ethane cup in LN<sub>2</sub> and then fill it with liquid ethane.
  - Confirm ethane is fully liquefied (not bubbling vigorously).
  - Place the ethane container into position in the CP3.
  - Ensure humidity and temperature in the room are within acceptable range.
- 

### Grid & Sample Preparation

- Prepare grids (glow-discharged if needed).
  - Load the grid onto the tweezers.
  - Set blotting time (e.g., 1–2 seconds depending on sample).
  - Apply 3–4 μL of sample to the grid.
- 

### Vitrification

- Insert the grid with tweezers into the CP3 arm.
  - Adjust alignment so grid is centered over blotting pads.
  - Press blot button to blot the grid.
  - Plunge the grid into liquid ethane immediately.
  - Transfer the vitrified grid into a pre-cooled cryo grid box under LN<sub>2</sub>.
- 

### Shutdown and Cleaning

- Store or transfer the cryo grid box to the cryo storage dewar.

- Let the ethane container return to room temperature in the fume hood.
- Empty LN<sub>2</sub> container and wipe dry.
- Clean tweezers and sample holders.
- Log the session (grid ID, sample, date, parameters used).