



Cryo-EM Merit Badge Standard Operating Procedure

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

Grid Clipping Standard Operating Procedure

version 1.1

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1. Purpose:

- 1.1. Successfully clip grids into TFS autogrid rings.

2. Definitions:

- 2.1. An autoloader system is a robotic system for loading cryo-EM grids into the microscope, in which the user inserts clipped grids into a cassette that is automatically loaded into the microscope using a retractable arm.
- 2.2. C-clips and rings are attached to a cryo-EM grid to allow a robotic retractable arm to grip and load the grid in an autoloader system.
- 2.3. Liquid Nitrogen (LN₂) is a cryogenic liquid stored under pressure.
- 2.4. Definition of terms for tools/equipment can be found in Figures 1 & 3.

3. Supplies & Equipment

- PPE (BSL-1)
 - Laboratory Coat
 - Nitrile Gloves
 - Goggles / Safety Glasses
 - Cryogenic Gloves
 - Face Mask
- Table-top clipping station with aluminum & brass transfer pedestal
- C-clips (one per grid)
- Autogrid rings (one per grid)
- Autogrid tweezer
- Fine tip tweezer
- Autogrid storage boxes and lids
- Autogrid box lid gripper tool
- C-clip clipping tool (one per grid)
- EM grids with your vitrified sample in a grid box under LN₂.
- Sample transfer dewar
- Liquid nitrogen storage dewar
- Screwdriver (if needed for screwed grid boxes)
- Large forceps
- Hair dryer or heat block (if needed to dry tools)

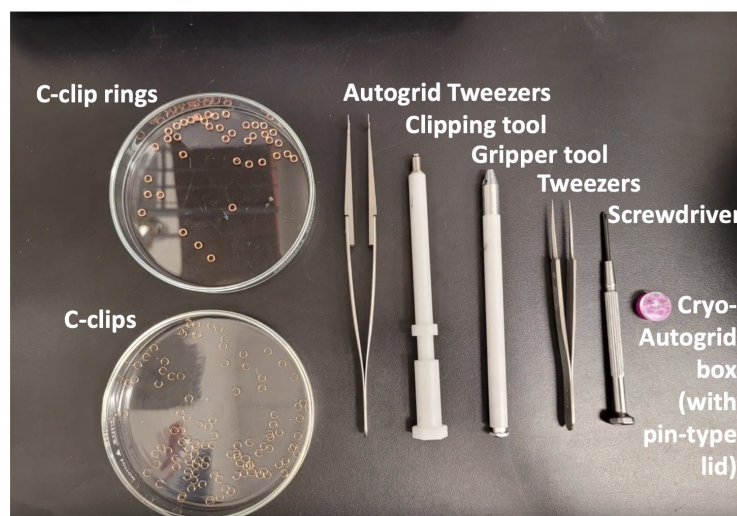


Figure 1. Tools needed for grid clipping.



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4. Procedure:

4.1. Loading C-clips into the clipping tool

4.1.1. Carefully pick up a C-clip with a fine tweezer and insert it into the open barrel of the clipping tool.

4.1.1.1. C-clips should be circular and not bent or distorted.

4.1.1.2. Do not re-use recovered C-clips or rings!

4.1.2. With tweezers, push the edge of the C-clip to one side of the c-clip tool barrel so that it is not perpendicular to the open end (Figure 2).

4.1.3. Holding the tool vertically, place the tip on a flat surface and gently press the top of the tool to push the C-clip toward the opening of the tool.

4.1.4. Visually inspect for proper placement of the C-clip. The C-clip should align with the inner rim of the barrel.

4.1.5. Repeat 4.1.1 to 4.1.4 to prepare a clipping tool for each grid that needs to be clipped.

4.1.5.1. If you need to re-use a cold C-clip tool, be especially sure to thoroughly warm it before using it again or else it may get stuck when you try and eject the C-clip. This can be done with a hair dryer or heat block.

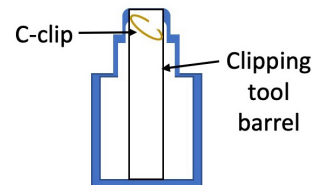


Figure 2. Cartoon of a cross section of the grid clipping tool barrel. C-clip should be inserted below the lip of the tool and can be at an angle, but not perpendicular to the flat surface you will use to push it to the barrel opening.

4.2. Preparing the Clipping Station

4.2.1. Assemble clipping station, with cover.

4.2.2. Fill the outer part of the chamber with liquid nitrogen and refill as needed until vigorous bubbling of LN2 has subsided.

4.2.2.1. During clipping, the LN2 should be at the upper lip of the aluminum portion of the transfer pedestal so that you clip in the LN2 vapor layer. You can also clip with the LN2 level above the aluminum such that you clip under liquid nitrogen if contamination from humidity is an issue, but this increases the chance of damaging the grid if liquid nitrogen is forcibly displaced during the clip ejection step.

4.2.3. Cool down all of the required tools and place grid boxes in the clipping station (as in Figure 3).

4.2.3.1. Using a tweezer, add C-rings to the brass pedestal to cool.

4.2.3.2. Place the prepared clipping tools along the notched area in the transfer station.

4.2.3.3. Transfer your cryo grid box with unclipped grids to the well of the aluminum pedestal.

4.2.3.4. Cool down a screwdriver and loosen the lid of the grid box.

4.2.3.5. Place a new autogrid storage box with pin type lid in the well of the aluminum pedestal.

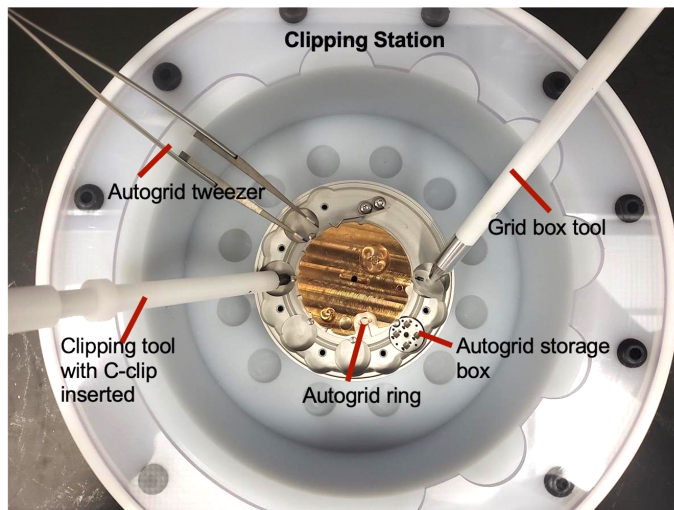


Figure 3. Clipping station with associated tools, labeled.



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- 4.2.3.6. Cool down a gripper tool and remove the lid of the pin type lid of the new grid box. You can screw the pin type lid into the pedestal between the grid boxes to secure them during clipping.

4.3. Clipping Grids

- 4.3.1. Using the autogrid tweezers, turn the brass top of the clipping station so that the grid loading pedestal nearest your boxes is open (loading position).
- 4.3.2. Transfer an autogrid ring, flat side down into loading pedestal open position (as shown in Figure 4).
- 4.3.2.1. Make sure that the “lip” on the ring is facing upwards, using magnification if necessary.
- 4.3.3. If you haven’t already, place a C-clip tool into the LN₂ to cool.
- 4.3.4. Using cold tweezers, carefully remove your grid from it’s box and place it in the center of the autogrid ring.
- 4.3.5. Using the autogrid tweezers, rotate the brass plate to the clipping position (the grid will be under the hole in the brass). This will allow for alignment of the clipping tool and the autogrid rim and grid.
- 4.3.6. optional: Touch the tip of the clipping tool against the top of the brass pedestal to remove the LN₂ from the tool, this helps to prevent the nitrogen from boiling during clipping, which can damage the carbon.
- 4.3.7. Insert the C-clip tool into the hole making sure the bottom is flat against the ring.
- 4.3.8. Depress the plunger to eject the C-clip.
- 4.3.9. Remove the clipping tool and look into the end of the barrel to ensure that the C-clip was released.
- 4.3.10. Rotate the brass top back to the grid loading position using the autogrid tweezers.
- 4.3.11. Inspect the grid to ensure it has clipped properly, using magnification if necessary (see figure 5). You can also flip the autogrid cartridge upside down and back to make sure the grid and/or clip ring do not fall out.
- 4.3.11.1. The C-clip should be lying flat and the grid should be flush.
- 4.3.11.2. Do not load if grid is torn or sticking up above the rim of the ring.
- 4.3.11.3. Improperly clipped grids can result in instrument downtime and therefore cannot be loaded onto the microscope.
- 4.3.12. Transfer the clipped grid to the autogrid box using the autogrid tweezers - **Ensure you have put the grid in the box in the correct direction specified by the center you are working at!!**
- 4.3.13. Repeat until all grids are clipped, adding LN₂ as needed.
- 4.3.14. Grids can be stored or loaded into the cassette.



Figure 4. Clip ring sitting on the pedestal with the lip facing up, and brass plate open to the pedestal. This is ready for placement of a grid onto the clip ring.

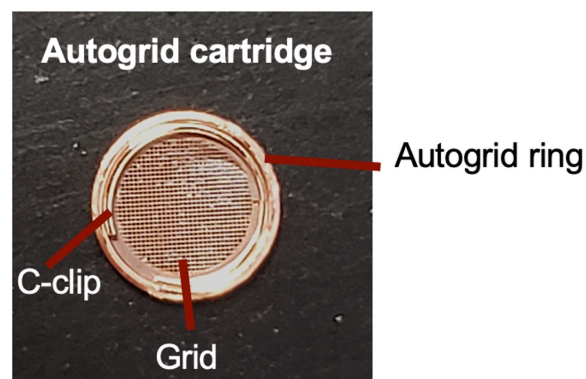


Figure 5. Properly clipped grid.



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4.4. Cleaning Up

- 4.4.1. If you aren't putting your grids directly into the transfer cassette, close and tighten the boxes containing your clipped grids. Place the clipped grid box in a transfer dewar to then store in its respective puck.
- 4.4.2. Remove all tools from the transfer station and allow them to warm up and dry.
- 4.4.3. Remove the aluminum and brass pedestals.
- 4.4.4. Empty the liquid nitrogen from the transfer station.
- 4.4.5. Return all tools to their respective locations.